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A PHYSIOLOGICAL STUDY OF SEASONAL CHANGES IN THE COMPOSITION OF THE PECAN DURING FRUIT DEVELOPMENT [1]

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LITERATURE REVIEW

Extensive studies of the morphological and anatomical development of the pecan nut (*Hicoria pecan* (Marsh.) Britton) [3] have been reported by J. G. Woodroof (*31*),[4] N. C. Woodroof (*35*), Woodroof and Woodroof (*32*, *33*, *34*), Shuhart (*26*, *27*, *28*, *29*), Isbell (*18*), and Adriance (*1*). No systematic physiological study of the normal process of nut development has been reported. In fact, the only data known to the writers relative to the physiological changes that occur during the development of pecan fruits are some analyses of pecan kernels made at intervals during the filling process by Woodroof and Woodroof (*33*).

Numerous investigations relating to the effects of cultural and climatic conditions on the final quantity and quality of oil in many oily seeds have been reported. Fewer studies of the course of development of the oil and its constituents in seeds during the entire period are available. These are represented by the studies of Garner, Allard, and Foubert (*15*), Eyre and Fisher (*10*), Eyre (*9*), Bushey, Puhr, and Hume (*5*), Bloomendaal (*3*), Dillman (*8*), Johnson (*21*), Fomin (*11*), and Fuchs (*12*), dealing chiefly with annual crop plants such as flax, cotton, and soybean. These workers found that oil formation usually occurs rapidly during a relatively short period and often is practically complete some time before the seeds are fully mature.

The most complete study of oil-seed metabolism seems to be that reported in 1912 by Ivanow (*19*), who determined changes in carbohydrates, oil, oil constants, and nitrogen fractions during the development of the seeds of flax, rape, hemp, poppy, and sunflower. In most cases the period of seed development was represented by only 3 or 4 sampling dates. In all cases there was a period of intensive oil formation occurring near the middle of seed development. This was accompanied by a simultaneous but apparently independent accumulation of protein in the seed. Reducing-sugar content invariably decreased during the time when samples were taken, while sucrose showed increases in total amount in some cases but as a rule decreased in percentage concentration on a dry basis. Insoluble carbohydrate fractions usually decreased in percentage of dry weight, but the

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[2] The writers are indebted to L. D. Romberg, of the Division of Fruit and Vegetable Crops and Diseases, for assistance in the nitrogen determinations.

[3] Synonym, *Carya pecan* Engl. and Graebn.

[4] Reference is made by number (italic) to Literature Cited, p. 119.

actual amounts increased during the period of active growth, then remained relatively constant. There was no indication that pentosans in these oily seeds functioned as reserve materials or potential sources of oil. The various seed receptacles, insofar as they were studied, were found to lose sugars and nitrogen, especially soluble nitrogen, and to gain in percentage of insoluble carbohydrates, during the ripening process. In general, these results were considered to establish glucose as the precursor of oil formation in oily seeds. However, the major interest in Ivanow's studies concerns the chemical properties of the oil during its formation.

Studies on the development of oily seeds, in which attention was paid to constituents other than oil, seem to be relatively scarce. Perhaps the earliest work is that of Müntz (*25*), who determined sugars, starch, oil, and protein in rape seed and rape hulls at seven different stages in the development of the seed. After most of the oil was formed starch decreased and sucrose increased in the seed. Protein accumulated gradually during the ripening period. Since there was a notable decrease in the sugar content of the hulls and no important change in their low lipide content, Müntz concluded that these sugars were the principal source of carbon for oil formation and that the transformation took place in the seed as fast as the sugars were translocated. He also pointed out that, in general, immature oily seeds contain both reducing and nonreducing sugars, while the mature seeds contain only nonreducing forms. Leclerc du Sablon (*22*, *23*) later presented data showing a decrease in the percentage of carbohydrate fractions in walnuts and almonds coincident with the increase in percentage of oil. However, he considered only the reducing-sugar fraction to undergo conversion to oil, since the absolute amounts of sucrose and of "amylose" were said to increase rather than decrease during the ripening process. Gerber (*16*, *17*) found a rise in respiratory quotient above 1 during oil formation in olives and castor beans, indicating a conversion of carbohydrate to oil. Vallée (*30*) studied changes in sugar and oil content of almond kernels and pericarp. Since most of his data were expressed only on a fresh-weight basis, his interpretations are questionable. A decrease in percentage of sugars in the kernel on a dry basis preceding and during oil formation was considered as evidence that both reducing sugars and sucrose served as sources of the oil.

More recently Gallup (*13*, *14*) and Caskey and Gallup (*6*) have studied quite extensively the chemical changes occurring in the cotton boll during its growth. Both the entire boll and the seeds showed gradual increases in the amounts of ash, crude protein, crude fiber, and oil from 16 days after blossoming until maturity. The percentage of nitrogen-free extract dropped rapidly, but, in terms of absolute amounts per boll or per seed, a similar decrease was not evident. In the lint there were decreases in percentage of all fractions except crude fiber, which increased rapidly. In terms of actual weight, nitrogen-free extract as well as crude fiber increased, while other constituents were irregular. Sugars determined from the twenty-first day to maturity, when oil formation was most active, decreased both in amount and in proportion to other substances in all parts of the boll. Lonzinger and Raskina (*24*), working with cottonseed, found a rapid increase in oil up to an age of 50 days.

Water-soluble material decreased for 35 days and then became constant; crude fiber increased for 40 days and then became constant; ash increased for 35 days and decreased after 50 days.

No study of the development of all major constituents in perennial oil-seed fruits, such as the pecan, has previously been reported. Such information on the physiological development of the pecan fruit is particularly desirable in order to understand fully the effects of various orchard conditions on the quality of the mature fruit or nut. A knowledge of the normal physiological development of various parts of the pecan fruit is necessary to an understanding of the effects of such factors as water shortage, defoliation by insect or fungus attack, shortage or abundance of mineral materials essential to the tree, and many other conditions that may develop in the orchard.

MATERIALS AND METHODS

Sample material was collected at intervals throughout the period from May 18 to November 14, 1932, from trees of the Burkett and Stuart varieties of pecan growing on the Pearce Farm near Austin, Tex. Table 1 gives a summarized description of the condition of the fruits on the various sampling dates, including the approximate number of weeks from full bloom and the average green weight per fruit. Samples collected on each date represented the average condition of the fruits for that date. All samples were taken from the same trees.

TABLE 1.—*Condition of pecan fruits on the several sampling dates*

BURKETT VARIETY

Sampling date	Time after full bloom	Average green weight per fruit	Condition of material
	Weeks	*Grams*	
May 18	5	0.075	Young nutlets with stigmatic surfaces quite black, hard, and dry.
June 30	11	1.99	Apparently same as on May 18 except for size.
July 21	14	7.69	Apparently same as on June 30 except for size.
Sept. 1	20	32.35	Shucks firmly attached to shells; shells beginning to harden; kernels very watery and low in dry weight.
Sept. 12	22	35.92	Shells quite hard; kernels not watery but very sticky.
Sept. 19	23	33.34	Kernels doughy; red packing material very sticky.
Sept. 26	24	38.50	Same as on Sept. 19.
Oct. 3	25	38.29	Shucks loose from shells but not split along the four rays; shell markings developed.
Oct. 10	26	38.09	Shucks loose from shells and many split along the four rays.
Oct. 17	27	34.40	Shucks practically all split along the four rays; red packing material dried out considerably and more easily separated from kernel than previously.
Oct. 19 to Nov. 14	27–31		Nuts apparently mature.

STUART VARIETY

May 18	5	0.078	Young nutlets with stigmatic surfaces quite black, hard, and dry.
June 30	11	2.58	Apparently same as on May 18 except for size.
July 21	14	8.68	Apparently same as on June 30 except for size.
Sept. 2	20	32.14	Shucks firmly attached to shells; shells quite hard; kernels not watery but very sticky.
Sept. 19	23	38.52	Kernels doughy; red packing material very sticky.
Sept. 26	24	46.06	Shucks beginning to loosen from shells.
Oct. 3	25	46.25	Shucks loose from shells and many split along the four rays; shell markings developed.
Oct. 10	26	42.70	Shucks practically all split along the four rays.
Oct. 17	27	38.87	Red packing material dried out considerably and more easily separated from kernels than previously.
Oct. 19 to Nov. 14	27–31		Nuts apparently mature.

SAMPLING METHODS

The nut clusters were picked as rapidly as possible during the forenoon and brought to the laboratory in paper bags. The individual fruits were picked from the clusters, counted, and the whole lot weighed in order to determine the average weight per fruit at each sampling date. The whole fruits were ground in a food chopper, and, after thorough mixing, portions were weighed out for samples to be preserved by drying or by boiling in 80-percent alcohol. Beginning September 1, samples of separate tissues or fruit parts were taken in addition to the whole-fruit samples. The shuck was peeled and scraped away from the shell. The nut was then cracked and the kernel removed as completely as possible. The remaining material constituted the shell sample. All possible precautions were taken to prevent loss of material while the separations were being made. Record was kept of the number of fruits used for each separation (usually 50) and of the total weight of each type of tissue. Finally, each tissue was ground in a food chopper or a Nixtamal mill and portions were weighed out as for the whole-fruit samples.

The samples to be dried were killed by placing them in an oven at 100° C. for 1 to 2 hours. The others were transferred immediately to 500 cc Erlenmeyer flasks containing about 1 g of calcium carbonate with sufficient hot 95-percent ethyl alcohol to give a final alcohol concentration of at least 80 percent. The flasks were then closed with short-stemmed funnels and heated for approximately 30 minutes on a water bath kept at 90° to 95° C. The flasks were stoppered while still hot and stored in the dark until the samples were used for sugar analyses.

The kernels of the September 1 samples of the Burkett variety were still too watery to permit cracking off the shell after removal of the shuck. In order to remove the kernels without loss of the fluid they contained, the shuck and shell together were cut away from the kernel with a sharp knife, since it was found that leaving the shuck attached to the shell facilitated the separation. A similar lot of nuts was used from which the shucks were removed and sampled. The remaining shell and kernel portion was ground as a whole and sampled. From this combination analytical values for the shell alone could be calculated.

Beginning with the samples of September 19, it became practically impossible to make a perfectly clean separation between kernel and shell because of the very sticky nature of the red packing material between the two tissues.

ANALYTICAL METHODS

Whenever the nature of the material permitted, the official methods of the Association of Official Agricultural Chemists (*2*) were employed.

DRY MATTER.—Samples that had been weighed into aluminum cans and placed in ovens at 100° C. for 1 to 2 hours were transferred to a vacuum oven and dried to constant weight at a temperature of 80° and a pressure of less than 1 mm of mercury. The resulting dry material was ground in a drug mill or food chopper and stored in 8-ounce glass bottles. Kernel samples were kept in a refrigerator at 0° to 5°, the others at room temperature. The material was redried under the same conditions before it was used in further analyses.

TOTAL NITROGEN.—The procedure followed closely the Kjeldahl-Gunning-Arnold method except for the use of copper wire as the digestion catalyst, and the use of 30 cc of sulphuric acid rather than 25 cc because of the high oil content of some of the samples. In general, 2-g portions of dried material were used except for the oily kernels, in which case 1 g was more suitable.

ASH.—Samples of 2 to 5 g of the dried material were weighed into ignited and tared crucibles. Ashing was carried out in an electric muffle in which the temperature was raised slowly to a dull red heat, where it was held until ashing was complete.

OIL.—Twenty-five-gram samples of dried material were weighed into 100-cc beakers and transferred to alundum thimbles, the adhering oil being removed with the petroleum ether (b. p. 30–60) used to fill the large-size Soxhlet extractors. Extraction was continued for 8 hours, which time had been shown to be sufficient for removal of oil from pecan kernels. After the solvent had been distilled off the flasks and contents were dried for 4 hours in an air oven at 100° C., cooled in desiccators, and weighed. Deiler and Fraps (*7*) have reported that pecan oil when heated in a steam oven gained only 0.65 percent of its weight in 4 days, and it has been the experience in this laboratory that any increases in weight of oil due to oxidation during the 4-hour drying periods are insignificant. Since preliminary extractions of shuck and shell tissues showed that they contained relatively small amounts of lipide, it was considered sufficient to determine the oil content only of the kernel and whole-fruit samples.

FREE FATTY ACIDS.—The ether extracts of the kernel samples, amounting as a rule to 17 to 19 g, were titrated with alkali according to the official methods (*2, p. 293* [*32*]). The results were calculated as percentage of oleic acid in the oil.

SUGARS.—The alcohol-preserved samples, representing as a rule 50 g of fresh material, were transferred to large Soxhlet extractors with 80-percent alcohol and extracted for 22 to 24 hours on a water bath kept at 95° to 99° C. The extracts of kernel and whole-fruit samples were transferred to separatory funnels, treated with 75 cc of chloroform, and diluted with water until a distinct chloroform layer separated. After standing overnight, the chloroform-lipide layer was run off and discarded. Such a chloroform extraction proved to be unnecessary for shell and shuck samples.

The alcohol extract was distilled almost to dryness in vacuo at temperatures not above 60° C. The residue was taken up with water, treated with sufficient saturated neutral lead acetate to clear the solution, and made up to a volume of 250 cc or 500 cc. Most pecan tissues require unusually large quantities of lead acetate for satisfactory clearing, 25 cc of saturated solution being necessary for all the samples except those of kernels, for which 10 cc was used. The cleared filtrate was deleaded with solid anhydrous disodium phosphate.

The Munson-Walker method was used for the determination of sugars in aliquots of the lead-free filtrate. Reduced copper was determined by the volumetric permanganate method. For the determination of total sugars, inversion was accomplished by means of acid at room temperature as directed in official methods (*2, p. 187,* [*23c*]).

Since previous work had shown that the sugar in mature pecan kernels is almost exclusively sucrose, all reducing sugar values were calculated as invert sugar, and nonreducing sugars as sucrose.

POLYSACCHARIDES.—The alcohol-insoluble residues were transferred to tared aluminum cans, dried in an air oven at 100° C., and weighed. After being ground in a drug mill they were stored in glass bottles and redried under the same conditions just before samples were weighed out for determination of polysaccharides. The dry residues from whole-fruit samples were extracted with petroleum ether to remove oil, and reweighed.

The official method for determination of starch in feeding stuffs by direct acid hydrolysis (*2, p. 119* [*21*]) was applied to all residues except those from the kernel samples. The preliminary cold-water extractions were eliminated, since sugars had already been extracted with alcohol. The method could not be used satisfactorily on residues from the kernel samples because the acid seemed to dissolve enough of the high-protein content to interfere with the precipitation of cuprous oxide. No such difficulty appeared in the case of the whole-fruit samples, so the values for the kernel portion were calculated by difference from the data for whole-fruit, shuck, and shell samples.

Since the iodine-potassium-iodide test gave negative results on all types of preserved tissues, and since the diastase method for starch gave insignificant values on the first two whole-fruit samples as well as on representative shuck samples, the latter method was not used as was originally intended.

ANALYTICAL DATA

The accumulation of dry matter in the pecan fruit and several of its parts is shown in table 2. It is evident that the shuck and shell portions were structurally almost complete at the time the first separations were made. The rest of the analytical data are presented graphically in figures 1 to 14, which are self-explanatory. In all graphs the data for each type of tissue are consistently represented by the same kind of point mark, and the same base line is used throughout the series. The harvesting date, October 18, comes at 153 in the scale of days from the first sampling. Samples were not collected on the date of harvest, but were taken on the preceding and following days, so that the flow of materials from the tree into the fruit was free to continue for 1 day after the last preharvest sample.

The marked differences between curves obtained from data calculated as percentage of dry weight and those calculated as weight per fruit illustrate the importance in work of this type of knowing actual amounts of material in each tissue as well as the mere ratios of materials that the usual percentage values represent.

The close similarity in the graphs for the two varieties makes any distinction between them practically unnecessary in most cases.

TABLE 2.—*Dry-weight changes in the pecan fruit*

BURKETT VARIETY

Date	Days	Average dry weight per fruit of—				Date	Days	Average dry weight per fruit of—			
		Whole fruit	Shuck	Shell	Kernel			Whole fruit	Shuck	Shell	Kernel
1932	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*1932*	*Number*	*Grams*	*Grams*	*Grams*	*Grams*
May 18	0	0.024				Oct. 17	152	14.58	4.55	4.41	6.22
June 30	43	.58				Oct. 19	154			4.04	5.39
July 21	64	1.99				Oct. 21	156			4.00	5.45
Sept. 1	106	8.27	3.61	3.91	0.54	Oct. 24	159			3.96	5.50
Sept. 12	117	10.52	4.29	4.14	1.46	Oct. 26	161			3.87	5.40
Sept. 19	124	11.23	4.60	4.06	3.39	Oct. 28	163			4.04	5.48
Sept. 26	131	13.24	4.31	4.31	4.38	Nov. 4	170			4.00	5.45
Oct. 3	138	13.92	4.33	4.14	5.58	Nov. 14	180			3.93	5.46
Oct. 10	145	15.01	4.42	4.28	6.12						

STUART VARIETY

May 18	0	0.025				Oct. 17	152	15.19	4.20	5.45	5.28
June 30	43	.68				Oct. 19	154			5.69	5.43
July 21	64	2.08				Oct. 21	156			5.67	5.16
Sept. 2	107	9.45	3.48	5.05	0.94	Oct. 24	159			5.65	5.27
Sept. 19	124	12.52	4.04	4.97	3.65	Oct. 26	161			5.77	5.33
Sept. 26	131	14.46	4.54	5.48	4.36	Oct. 28	163			5.70	5.17
Oct. 3	138	14.53	4.40	5.21	4.84	Nov. 4	170			5.82	5.37
Oct. 10	145	14.75	4.19	5.19	5.18	Nov. 14	180			5.82	5.38

MOISTURE AND DRY MATTER

Figures 1 and 2 show that the kernel and shell portions of the fruit were subject to a dehydration process from the time the watery kernel could first be removed whole until it was mature, although the surrounding shuck contained more than 75 percent moisture throughout the entire period. The increase in percentage of dry matter in kernel and shell followed roughly a double drying curve; the first, from September 1 until harvest; the second, from harvest on.

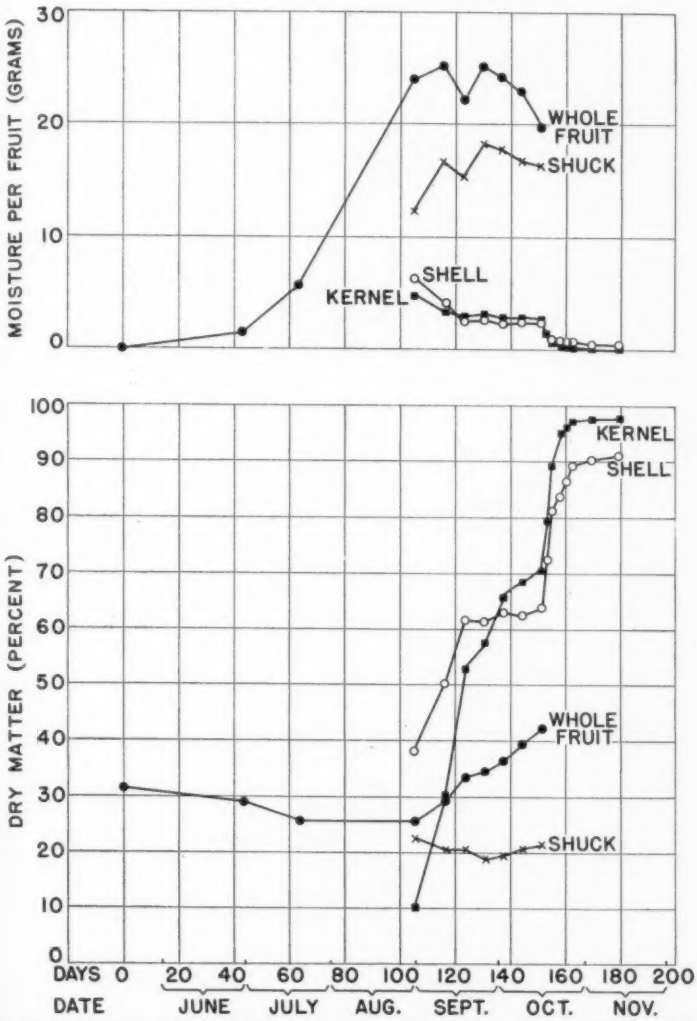

FIGURE 1.—Changes in dry matter and moisture content during development of the pecan nut (Burkett variety).

ASH

The amount of mineral matter in the whole fruit increased quite regularly from the beginning of its development until harvest. It is

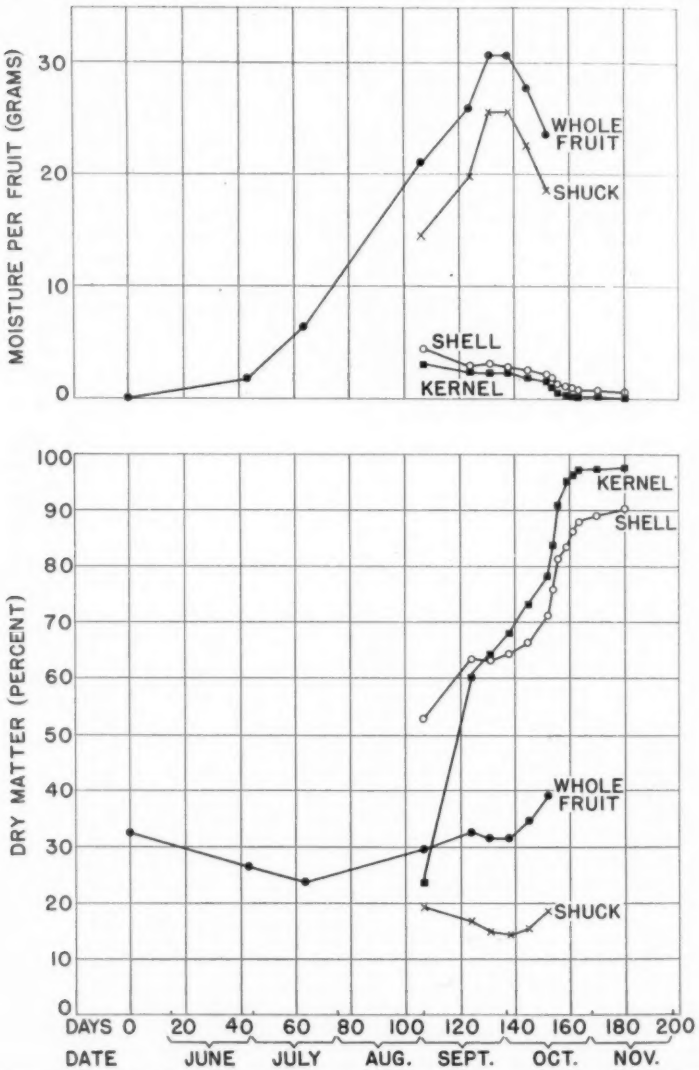

FIGURE 2.—Changes in dry matter and moisture content during development of the pecan nut (Stuart variety).

significant that the larger part of this accumulation occurred in the shuck, where the mineral constituents doubled in amount during the

relatively short filling period. The decrease in percentage of ash (dry weight) in the whole fruit and in the kernel was due to dilution with other materials, e. g., structural carbohydrates and oil laid down

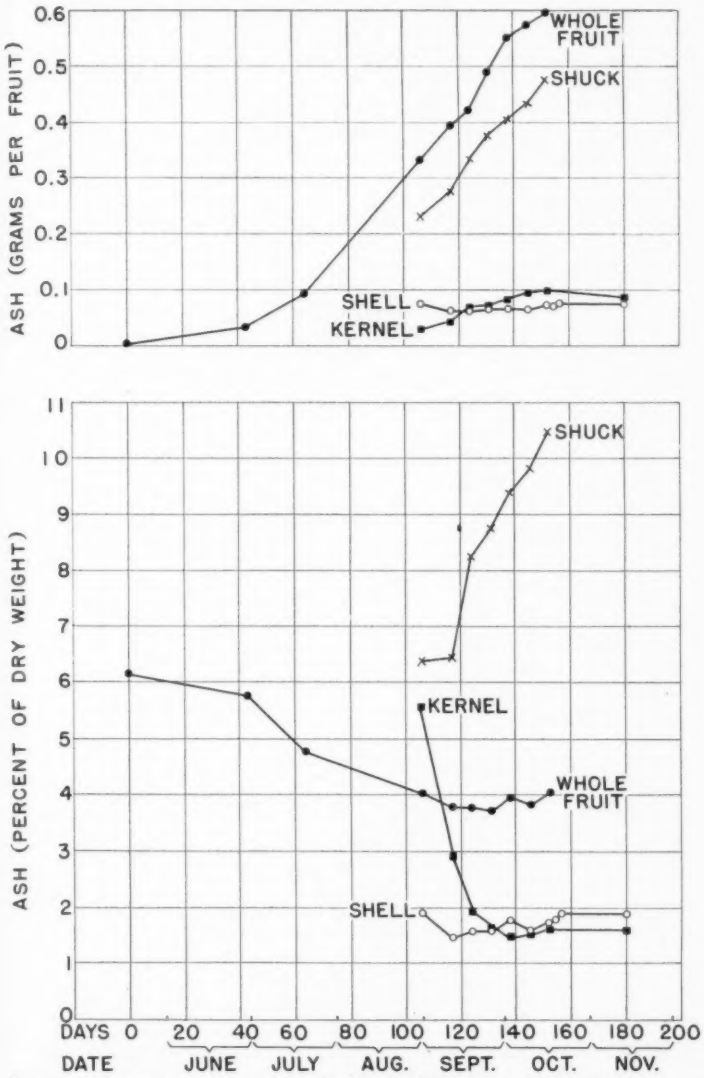

FIGURE 3.—Changes in ash content during development of the pecan nut (Burkett variety).

during that time, and not to any loss of mineral constituents. The mineral constituents contained in the kernel showed a gradual increase as filling progressed. All these changes are illustrated in figures 3 and 4.

TOTAL NITROGEN

Figures 5 and 6 show that the nitrogen content of the pecan fruit increase throughout the growing period, although the concentration of nitrogen on a dry-weight basis decreased until filling had begun.

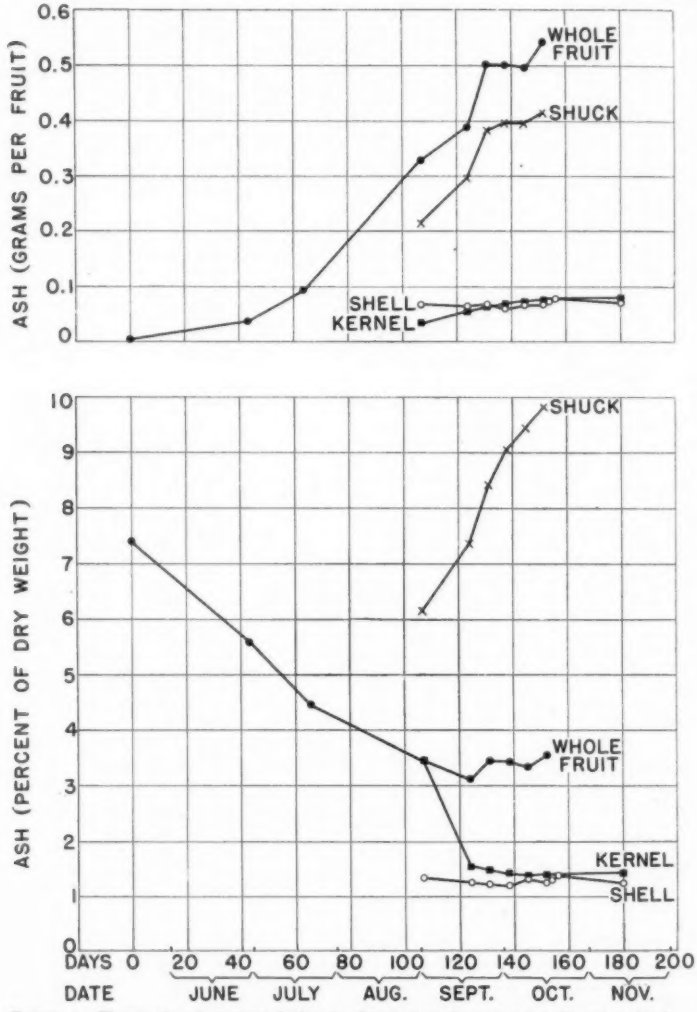

FIGURE 4.—Changes in ash content during development of the pecan nut (Stuart variety).

During the filling process, the nitrogen content of shucks and shells remained practically constant and the increase of nitrogen in the whole fruit was due almost entirely to the deposition of kernel protein. The latter process was very nearly completed during September.

OIL

Figures 7 and 8 show the course of oil formation in the pecan kernel. On September 1 oil synthesis had barely begun, but during the next 30 days 75 to 80 percent of the final oil content of the kernel was laid down. By September 19 the oil in the kernels had reached a concentration of 70 percent on a dry-weight basis, after which time there was a gradual increase until harvest, when the Burkett variety contained about 74 percent of oil and the Stuart variety about 76 percent, on a dry-weight basis. On a green-weight basis the concentration of oil

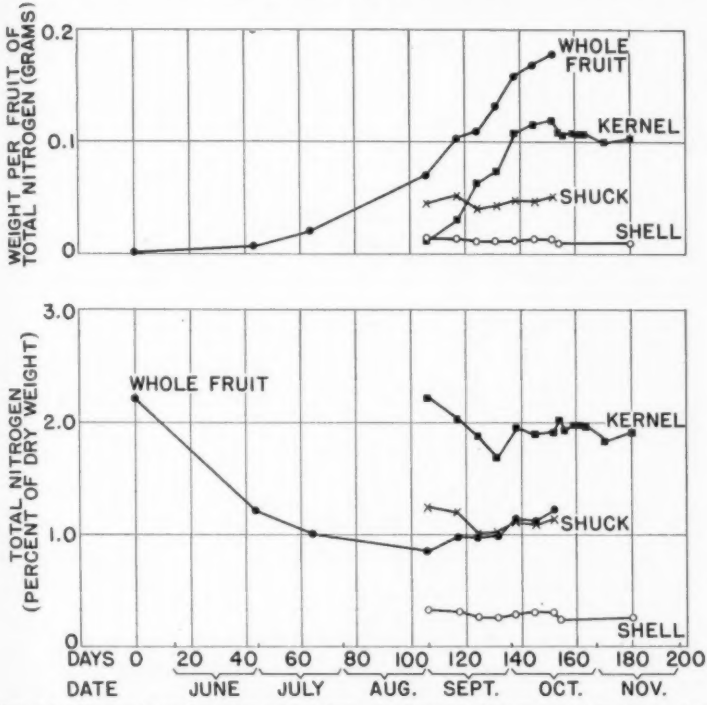

FIGURE 5.—Changes in total nitrogen content during development of the pecan nut (Burkett variety).

in the kernels at harvest was near 50 percent in the Burkett and 57 percent in the Stuart, and it increased rapidly for a few days following harvest. However, this increase was due entirely to the loss of moisture from the nuts, since there was no significant change in the actual amount of oil present in the kernel or in the concentration of oil on a dry-weight basis.

The actual amount of oil per nut increased from the time filling began until harvest and remained relatively constant thereafter. The sudden drop at harvest time in the case of the Burkett variety is almost certainly a result of the necessity of changing to a new group of trees for after-harvest samples.

The values for free fatty acids in the oil are not given, because they were insignificantly low and showed no definite trend. The oil from the earliest kernel sample was highest, with a value of 0.7 percent, but the small amount of oil available made the error of determination very great. All subsequent values were less than 0.25 percent. In general, the data indicate no real tendency toward accumulation of free fatty acids prior to glyceride formation.

SUGARS

In considering the results of sugar analyses it seems adequate to discuss only total sugar values, since for any one tissue practically all

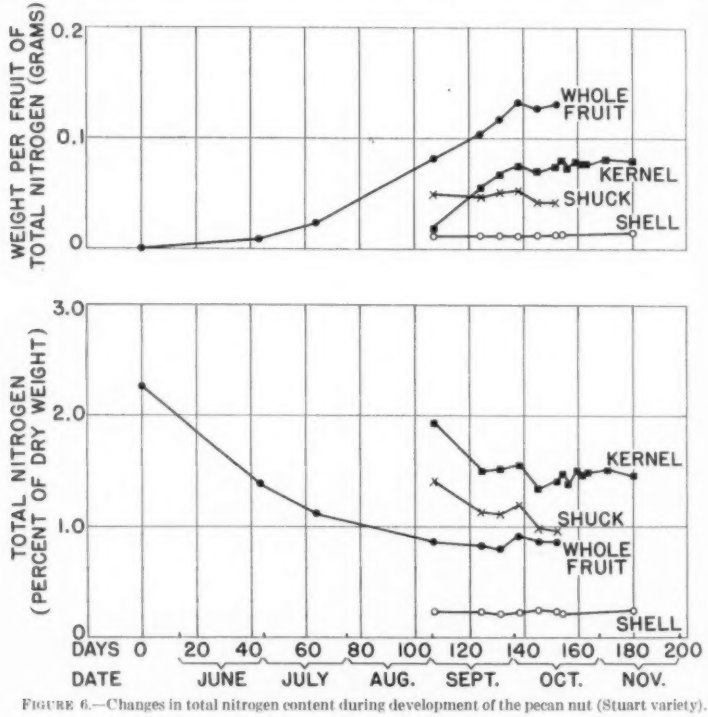

FIGURE 6.—Changes in total nitrogen content during development of the pecan nut (Stuart variety).

of the sugar was found either in the reducing or in the nonreducing form and no definite shift from one to the other was observed. Except in the very early watery stage the kernel contained almost exclusively nonreducing sugar, all indications being that it was sucrose. In other tissues reducing sugar was the predominant form.

Figures 9 and 10 show the changes in sugar values during the whole period of fruit formation. The sugar concentration in the whole fruit on a dry-weight basis rose to a sharp maximum near the middle of July, and thereafter decreased steadily until early October, when it remained relatively constant. On the weight-per-fruit basis, however, the maximum sugar content was not reached until the first part

of September. During the period of rapid production of oil and protein in the kernel there was a significant drop in the sugar content of the whole fruit.

In the shucks, both the sugar content per shuck and the sugar concentration on a dry-weight basis rose from September 1 until the formation of oil and protein were well under way, then dropped sharply.

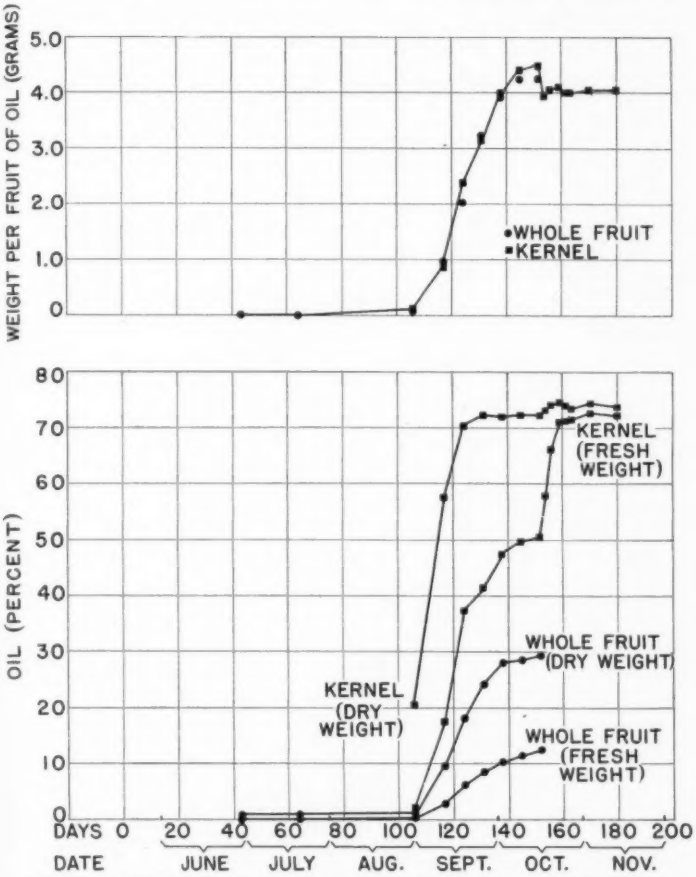

FIGURE 7.—Changes in oil content during development of the pecan nut (Burkett variety).

The relatively high sugar content of the early shell samples decreased rapidly during the first 2 weeks of September, remaining at a very low level thereafter.

Only one sample of the kernel in the watery stage was obtained, and it contained a very high concentration of sugar on a dry-weight basis, although the actual amount of sugar per nut was relatively small. The sugar concentration on a dry-weight basis as well as the amount per kernel dropped to a very low level at the time oil synthesis began and

remained low until most of the oil had been formed. Then there was a very rapid accumulation of nonreducing sugar until harvest. Of special interest is the fact that more than 50 percent of the sugar content of the mature kernel was laid down in the 2 weeks immediately preceding harvest. No definite influx of sugar into the whole fruit from the trees was observed during this period, but there was a drop

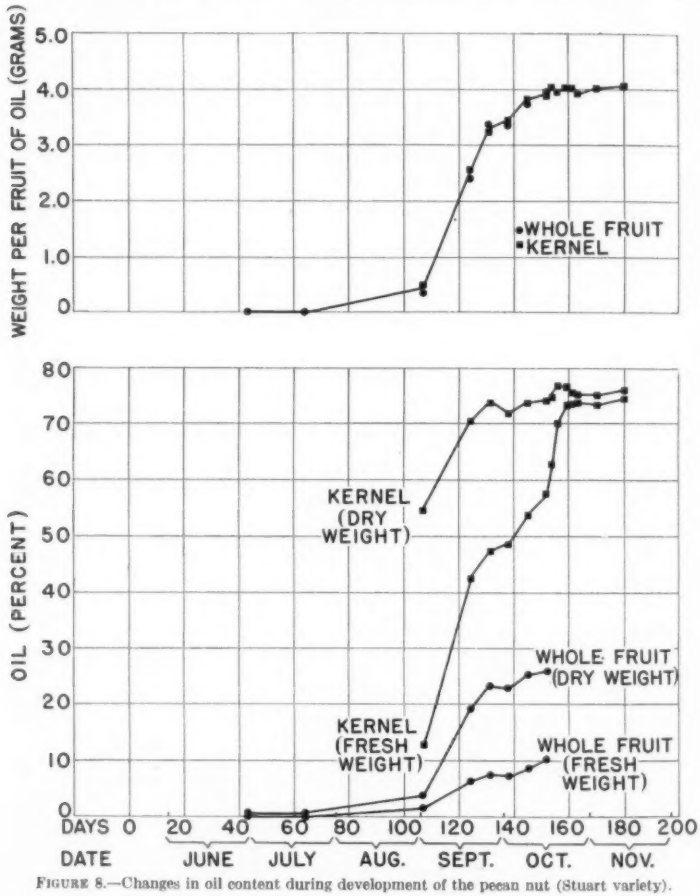

FIGURE 8.—Changes in oil content during development of the pecan nut (Stuart variety).

in the sugar content of the shuck corresponding roughly in amount to the increase in the kernel.

Figures 11 and 12 show the changes in the ratio of total sugar to moisture content. Assuming that all the sugar was dissolved in the portion of the water that was removed by the procedure used for moisture determinations, this should give an approximation of the concentration of sugar solution in the tissues. Calculated in this manner, the sugar concentrations show surprisingly small variations

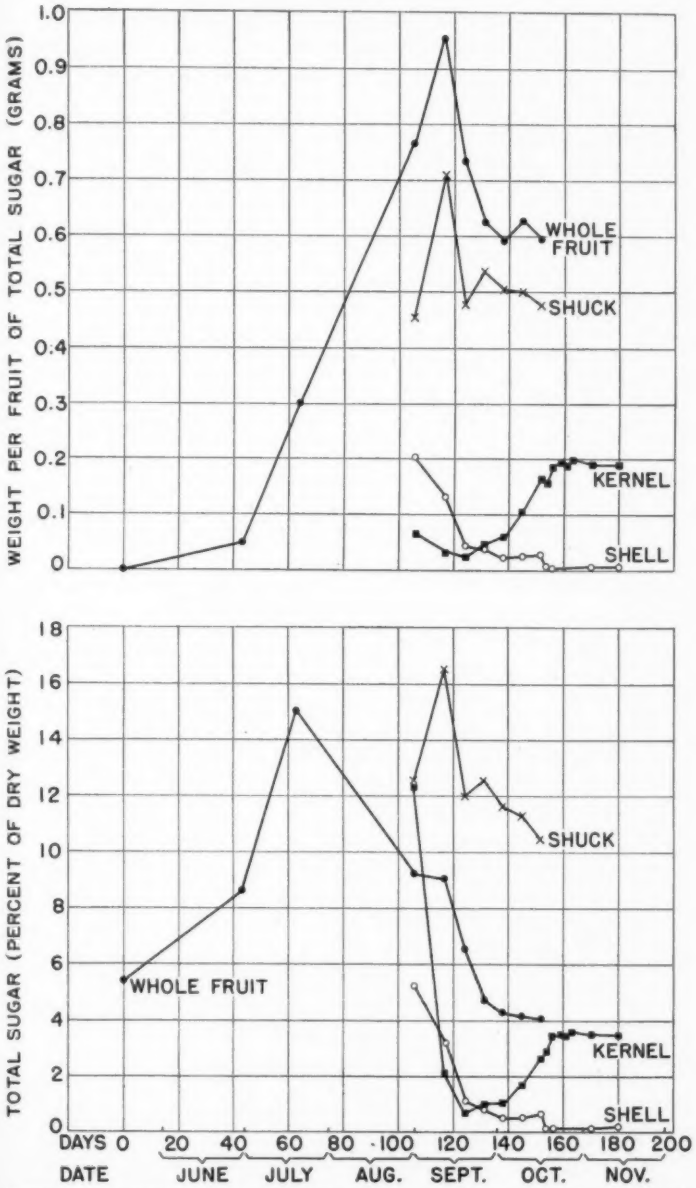

FIGURE 9.—Changes in total sugar content during development of the pecan nut (Burkett variety).

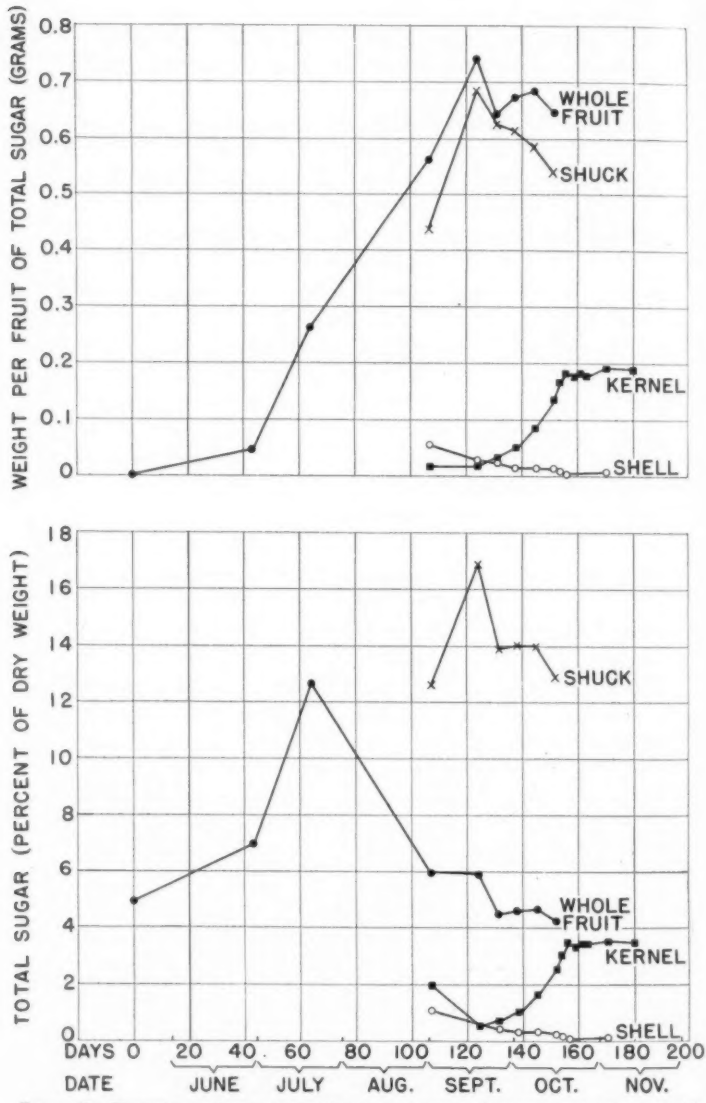

FIGURE 10.—Changes in total sugar content during development of the pecan nut (Stuart variety).

except for the kernel tissues. Although the major portion of the increase in the ratio for kernel tissues occurred as a result of dehydration after harvest, the curve had started definitely upward by the

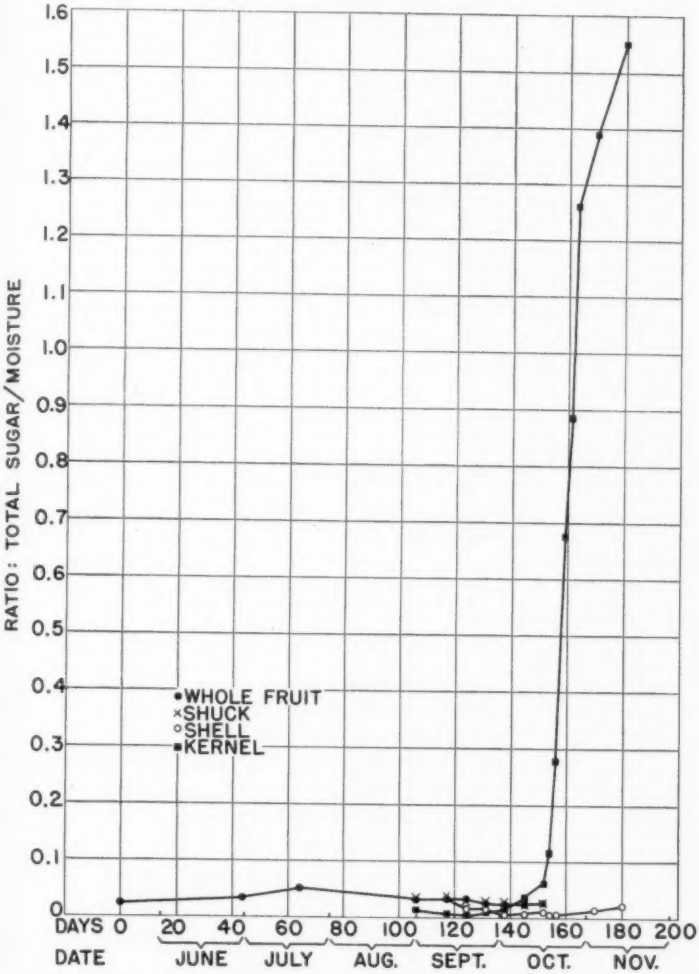

FIGURE 11.—Ratio of total sugar to moisture during the development of the pecan nut (Burkett variety).

middle of September, when syntheses of oil and of protein were at their height.

POLYSACCHARIDES

The changes in acid-hydrolyzable polysaccharides are shown in figures 13 and 14. These substances increased rapidly in the whole

fruit during the summer, when shell and other structural parts were forming, and more slowly after filling of the kernel began. From the time tissue separations were first made, the acid-hydrolyzable polysaccharides of shuck and shell portions showed no important change.

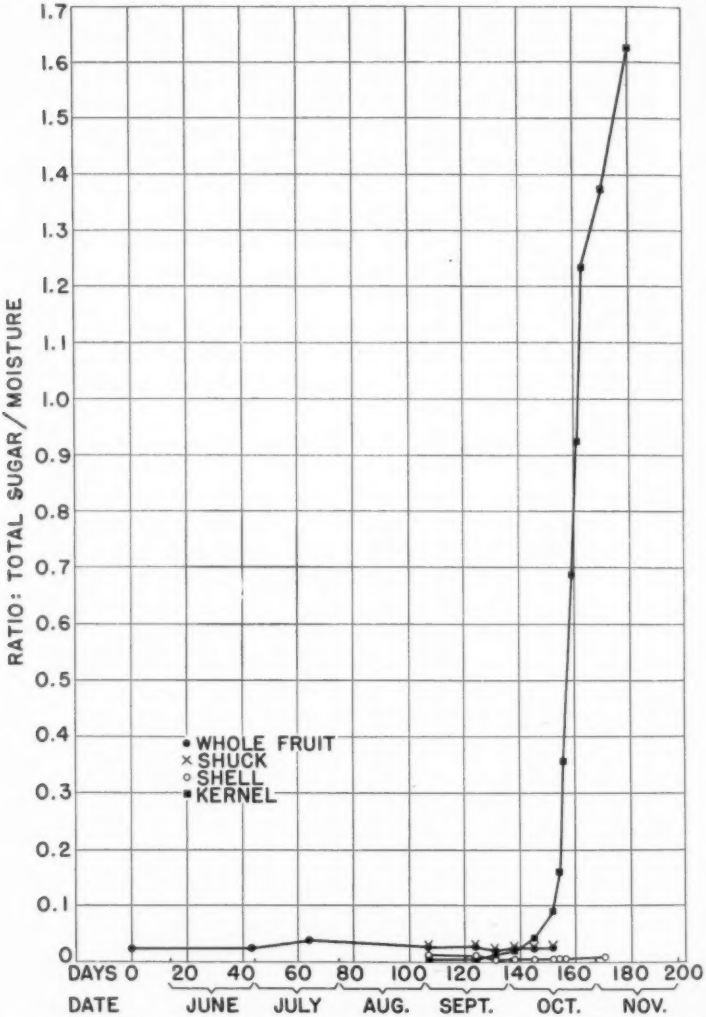

FIGURE 12.—Ratio of total sugar to moisture during the development of the pecan nut (Stuart variety).

Certainly they were not functioning as carbohydrate reserves from which the sucrose so rapidly deposited in the kernel could be derived.

The acid-hydrolyzable polysaccharides in the kernel increased gradually during the filling process. Since these values had to be

calculated as the difference between the whole-fruit data obtained with one lot of 50 nuts and the shuck plus the shell data obtained with a second lot of 50 nuts, any difference in the two lots due to sampling error would show up in the kernel value. This may explain the position of the last point on the Burkett kernel curve.

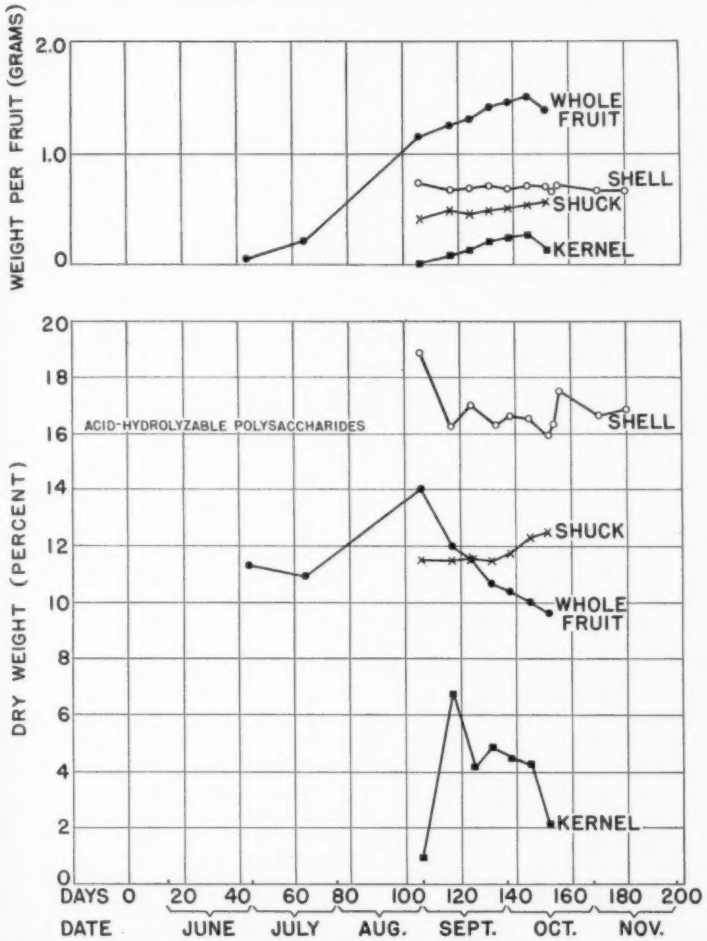

FIGURE 13.—Changes in acid-hydrolyzable polysaccharide content during the development of the pecan nut (Burkett variety).

GENERAL DISCUSSION

For present purposes, the development of the pecan fruit may be divided into two more or less distinct periods. The first comprises the time from blossoming until the kernel begins to fill, i. e., from early May to late August or early September in central Texas. The second

period covers the processes of filling and ripening of the nut, the term "filling" referring to building up of actual dry-matter content of the kernel.

Because of the large number of fruits necessary for an adequate sample, the number of samplings during the first period was necessarily limited and separation into various parts was impracticable. The data obtained show that on a weight-per-fruit basis large increases in all

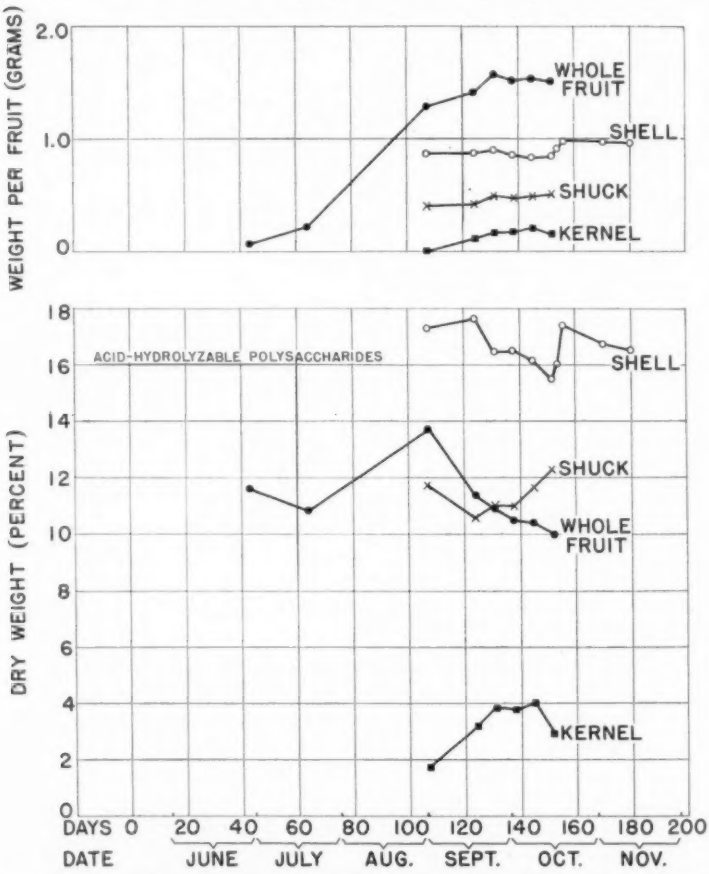

FIGURE 14.—Changes in acid-hydrolyzable polysaccharide content during the development of the pecan nut (Stuart variety).

the determined constituents except oil occurred during this time. In the case of total nitrogen and mineral constituents, however, the increase was not so rapid as that of structural materials in general, so that the percentage of nitrogen and ash on a dry-weight basis showed a fairly regular decrease. The sharp maximum in the concentration of total sugar occurring in July may be related to the observations of Woodroof and Woodroof (*31*, *33*, *34*, *35*) that the fertilized egg begins

to undergo division at about that time. The rise in percentage of acid-hydrolyzable polysaccharides during August was very probably the result of the later stages of shell formation.

After the beginning of the filling process about September 1, the amount of ash in the shell and of total nitrogen and acid-hydrolyzable polysaccharides in both shell and shuck remained practically constant. On the other hand, mineral constituents, protein, oil, and acid-hydrolyzable polysaccharides began to accumulate in the kernel at a rapid rate, and deposition of these substances continued until harvest. The major portion of each of these constituents, however, accumulated in the kernel as early as October 1.

These results agree in every essential with the data given by Woodroof and Woodroof (*33*). However, their conclusions that the decrease in percentage of nitrogen-free extract was due to a decrease in soluble carbohydrates and that the increase in percentage of oil was due to transformation of carbohydrate to oil appear to the writers not to be justified by the data presented, since the actual amount of nitrogen-free extract in the kernel in grams did not decrease but instead more than doubled during the period in question. Moreover, the recorded decreases in percentage of ash, protein, and fiber, as well as nitrogen-free extract, are largely the results of dilution with oil formed during the period, and represent actual increases in the amounts of these substances present in the kernel. The introduction into the kernel of 60 to 70 percent of its final weight as oil during the course of 4 to 6 weeks makes the drawing of conclusions from percentage composition data alone unusually hazardous.

The idea apparently becoming prevalent in the literature that the kernel of the pecan first fills with carbohydrate material which rapidly changes over to oil during the ripening process is entirely without a quantitative basis and is not borne out by the present study. The decrease in sugar content of the whole fruit during the period of oil synthesis, which is of greater magnitude than the decrease of sugar in any part, could account for not over 5 percent of the final oil. The highest total amount of sugar found at any time could not make more than 12 percent of the normal oil content. The acid-hydrolyzable polysaccharide fraction, which should be next in point of availability, shows no significant decrease in any part, and the whole amount in the kernel itself could not possibly make 3 percent of the final oil content. As a matter of fact, a simple calculation shows that 1 g of glucose contains only sufficient carbon to make 0.517 g of triolein, which, according to Boone (*4*) and Jamieson and Gertler (*20*), constitutes about 80 percent of pecan oil. If it be assumed that the energy difference between carbohydrate and fat also comes from combustion of glucose, as is probably the case, then the figure becomes only 0.368 g triolein from each gram of glucose as a maximum limit. By the first of September, when oil production was just beginning, the average dry weight of the whole fruit was 8.27 g for the Burkett variety and 9.45 g for the Stuart variety. If this entire dry weight had been pure glucose, and if it were completely changed into oil during ripening, it could not possibly have given more than 9.45×0.517 or 4.88 g of oil, and the more probable maximum would have been 9.45×0.368 or 3.48 g. Actually, both varieties produced very nearly 4 g of oil per fruit after September 1 and during the same period showed an increase in average dry weight of approximately 6 g.

Both the present data and simple calculations of the relationships involved leave no doubt that practically all of the oil content of the pecan kernel is formed from materials brought into the fruit from other parts of the tree at the time of oil formation. Whether the source of this material is reserve carbohydrates in twigs, trunk, or roots, or whether it is photosynthetic activity during the filling period, remains to be determined. There is no reason to suspect that a similar situation does not exist in the case of most of the protein content of the kernel. At any rate the nitrogen portion of it is definitely shown to be brought into the fruit from the tree at the time of filling.

It was interesting to note that the first samples in which dehiscence between the shuck and the shell was apparent (Oct. 3 for the Burkett variety and Sept. 26 for the Stuart variety) came at points on the curves where the accumulation of the protein and oil began to slow down. These are also the points where the rapid rise in sugar content of the kernel started.

The very marked increase in ash retained in the shuck as compared with that found in the kernel during the filling period may be due merely to an accidental screening out of mineral constituents by the shuck from the relatively large volume of plant sap which must pass through it on the way to the kernel. But whether accidental or not, such a large increase in mineral salts might conceivably have effects on the colloidal state of shuck tissues sufficient to suggest a causative mechanism for the dehiscence between shuck and shell and along the parenchymatous rays of the shuck, which occurs at maturity. Whether or not the latter phenomena have any causal relationship to the filling process, so that studies with a view toward controlling them would be of practical importance, remains to be determined.

The fact that the nonreducing sugar content of the pecan kernel was built up rapidly only after formation of the other constituents had almost stopped indicates that it is a storage form coming from the sugar entering the kernel in excess of that needed for synthesis of oil, protein, etc. Since no significant influx of sugar from the tree into the fruit was observed during the 2 weeks preceding harvest, and since there was a corresponding drop in sugar content of the shucks, it seems probable that most of the sugar content of the mature kernel was the result of translocation from the shuck during the later stages of maturity.

Since the sugar content of the pecan contributes to its quality and flavor, the foregoing results have practical significance in showing that harvesting too early is detrimental, and that possibly, from the quality standpoint, harvesting later than usual might be desirable. It is hoped that this point can be checked when the next season's crop becomes available. Evidence was not obtained to show whether or not a similar sugar increase would occur in nuts separated from the shuck.

It must be considered also that, while the greater amount of oil was synthesized in the kernel several weeks before ordinary harvest time, the amount of oil formed in the kernels during the later period is of enough importance to make it inadvisable to harvest before the nuts are ready to fall from the shucks. A similar situation holds, perhaps to a lesser extent, for the protein content of the nut.

The relatively short time during which the greater amounts of oil and protein were synthesized indicates a critical period in the filling

of nuts. It is probable that proper cultural practices immediately preceding and during this period may aid considerably in filling out a pecan crop when existing natural conditions happen to be unfavorable.

SUMMARY AND CONCLUSIONS

A thorough analytical study has been made of the physiological development of the pecan fruit and several of its parts from the time of flowering until the fruit is mature.

The two varieties used, Stuart, of eastern origin, and Burkett, of western origin, but both grown in central Texas, gave very nearly identical results.

A first period of growth from blossoming until late August or early September was characterized chiefly by formation of structural elements of the shuck and the shell. During the second period filling of the kernel was of major importance.

Most of the oil, protein, mineral, and acid-hydrolyzable polysaccharide content of the kernel developed during September. It is shown that practically all of the material from which oil is formed must be brought in from outside the fruit during the filling period. The same is probably true of protein also.

Very nearly the whole sugar content of the kernel, which is almost exclusively in a nonreducing form, appeared during the first half of October.

The data indicate that much of the sugar in the mature kernel may be a result of translocation from the shuck during the later stages of maturity.

Harvesting pecans before they are ready to fall from the shucks would seem to be inadvisable from the viewpoint of the quality of the nut.

A critical period in the filling of pecan nuts under central Texas conditions seems to occur during September.

LITERATURE CITED

(1) Adriance, G. W.
1931. factors influencing fruit setting in the pecan. Bot. Gaz. 91: 144–166, illus.

(2) Association of Official Agricultural Chemists.
1925. official and tentative methods of analysis. Compiled by the Committee on Editing Methods of Analysis. Revised to July 1, 1924. Ed. 2, 535 pp., illus. Washington, D. C.

(3) Bloomendaal, H. N.
1925. preliminary paper on the ripening of oilpalm-fruits. Commun. Gen. Expt. Sta. Alg. Ver. Rubberplanters Oostkust Sumatra, Gen. Ser. 20, 43 pp., illus. [Abstract in Expt. Sta. Rec. 57: 141. 1927.]

(4) Boone, P. D.
1924. chemical constituents of pecan oil. Indus. and Engin. Chem. 16: 554.

(5) Bushey, A. L., Puhr, L., and Hume, A. N.
1927. a study of certain physical and chemical characteristics of flaxseed and of linseed oil. S. Dak. Agr. Expt. Sta. Bull. 228, 11 pp.

(6) Caskey, C., Jr., and Gallup, W. D.
1931. changes in the sugar, oil, and gossypol content of the developing cotton boll. Jour. Agr. Research 42: 671–673.

(7) Deiler, A. C., and Fraps, G. S.
1910. pecan oil. Amer. Chem. Jour. 43: 90–91.

(8) Dillman, A. C.
1928. daily growth and oil content of flaxseeds. Jour. Agr. Research 37: 357–377, illus.
(9) Eyre, J. V.
1931. notes on oil development in the seed of a growing plant. Biochem. Jour. 25: [1902]–1908.
(10) ——— and Fisher, E. A.
1915. some considerations affecting the growing of linseed as a farm crop in England. I. variations in the oil content. Jour. Agr. Sci. [England] 7: [120]–134, illus.
(11) Fomin, A.
1929. the oil content in the seeds of oil-bearing plants. Masloboino Zhirovoe Delo no. 6, pp. 37–42. [Abstract in Chem. Abs. 26: 1815. 1932.]
(12) Fuchs, L.
1932. examination of fructus papaveris at various stages of growth. Pharm. Monatshefte 13: 223–225. [Abstract in Chem. Abs. 27: 561–562. 1933.]
(13) Gallup, W. D.
1927. the gossypol content and chemical composition of cotton-seeds during certain periods of development. Jour. Agr. Research 34: 987–992.
(14) ———
1928. a chemical study of the development of cotton bolls and the rate of formation of gossypol in the cotton seed. Jour. Agr. Research 36: 471–480.
(15) Garner, W. W., Allard, H. A., and Foubert, C. L.
1914. oil content of seeds as affected by the nutrition of the plant. Jour. Agr. Research 3: 227–249.
(16) Gerber, C.
1897. étude de la transformation des matières sucrées en huile dans les olives. Compt. Rend. Acad. Sci. [Paris] 125: 658–661.
(17) ———
1897. recherches sur la formation des réserves oléagineuses des graines et des fruits. Compt. Rend. Acad. Sci. [Paris] 125: 732–735.
(18) Isbell, C. L.
1928. growth studies of the pecan. Ala. Agr. Expt. Sta. Bull. 226, 68 pp., illus.
(19) Ivanow, S. L.
1912. über den stoffwechsel beim reifen ölhaltigen samen mit besonderer berücksichtigung der ölbildungsprozesse. Bot. Centbl. Beihefte (1) 28: [159]–191, illus.
(20) Jamieson, G. S., and Gertler, S. I.
1929. pecan oil. Oil & Fat Indus. 6 (10): 23–24.
(21) Johnson, I. J.
1932. the relation of agronomic practice to the quantity and quality of the oil in flaxseed. Jour. Agr. Research 45: 239–255, illus.
(22) Leclerc du Sablon.
1896. sur la formation des réserves non azotées de la noix et de l'amande. Compt. Rend. Acad. Sci. [Paris] 123: 1084–1086.
(23) ———
1897. sur les réserves oléagineuses de la noix. Rev. Gén. Bot. 9: 313–317.
(24) Lonzinger, E., and Raskina, R.
1931. a study of cottonseed and of cottonseed oil at various stages of maturity. Masloboino Zhirovoe Delo nos. 2–3, pp. 57–60; Chem. Indus. 27: 636, 1932. [Abstract in Chem. Abs. 26: 3127, 1932.]
(25) Müntz, A.
1886. recherches chimiques sur la maturation des graines. II. graines oléagineuses. Ann. Sci. Nat., Bot. (7) 3: 68–74.
(26) Shuhart, D. V.
1927. the formation and development of the pistillate flowers of the pecan. Okla. Agr. Expt. Sta. Bull. 163, 7 pp., illus.

(27) SHUHART, D. V.
1927. THE MORPHOLOGICAL DIFFERENTIATION OF THE PISTILLATE FLOWERS OF THE PECAN. Jour. Agr. Research 34: 687–696, illus.
(28) ———
1932. ENDOSPERM AND EMBRYO DEVELOPMENT AS RELATED TO FILLING OF PECANS AND WALNUTS. Amer. Soc. Hort. Sci. Proc. (1931) 28: 161–163, illus.
(29) ———
1932. MORPHOLOGY AND ANATOMY OF THE FRUIT OF HICORIA PECAN. Bot. Gaz. 93: 1–20, illus.
(30) VALLÉE, C.
1903. SUR LA PRÉSENCE DU SACCHAROSE DANS LES AMANDES ET SUR SON RÔLE DANS LA FORMATION DE L'HUILE. Compt. Rend. Acad. Sci. [Paris] 136: 114–117.
(31) WOODROOF, J. G.
1926. THE FRUIT-BUD, THE FLOWER, AND THEN THE PECAN NUT. Natl. Pecan Growers' Assoc. Proc. 25: 81–92, illus.
(32) ——— and WOODROOF, N. C.
1926. FRUIT-BUD DIFFERENTIATION AND SUBSEQUENT DEVELOPMENT OF THE FLOWERS IN THE HICORIA PECAN. Jour. Agr. Research 33: 677–685, illus.
(33) ——— and WOODROOF, N. C.
1927. THE DEVELOPMENT OF THE PECAN NUT (HICORIA PECAN) FROM FLOWER TO MATURITY. Jour. Agr. Research 34: 1049–1063, illus.
(34) ——— and WOODROOF, N. C.
1929. FLOWERING AND FRUITING HABIT OF THE PECAN. Natl. Pecan Growers' Assoc. Proc. 28: 128–140.
(35) WOODROOF, N. C.
1928. DEVELOPMENT OF THE EMBRYO SAC AND YOUNG EMBRYO OF HICORIA PECAN. Amer. Jour. Bot. 15: 416–421, illus.

A TETRAPLOID HYBRID OF MAIZE AND PERENNIAL TEOSINTE [1]

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INTRODUCTION

In a progeny of seven hybrid plants that resulted from pollinating waxy maize (*Zea mays* L.) with perennial teosinte (*Euchlaena perennis* Hitchc.) there appeared one plant with 40 chromosomes instead of the usual 30 chromosomes. The character of the plant and the behavior of its progeny compel the belief that this plant possessed two sets of maize chromosomes together with the two sets normal to perennial teosinte. This paper is a discussion of the contribution made by this plant and its progeny to an understanding of the synaptic attractions of homologous chromosomes in maize-teosinte hybrids.

Annual teosinte (*Euchlaena mexicana* Schrad.) resembles maize in having 10 pairs of chromosomes. That each of the 10 teosinte chromosomes has its homologue in maize seems certain. The two forms are completely interfertile, and in F_1 pollen mother cells meiosis is normal. Perennial teosinte (*E. perennis*) has 20 pairs of chromosomes. It seems clear that perennial teosinte is tetraploid in nature, having two sets of 10 pairs of chromosomes homologous to those of the annual teosinte and maize. This follows from the fact that genes located in a number of the maize chromosomes have been show to have two allelomorphs in perennial teosinte (*2*).[2]

Randolph's (*8*) experiments give even more convincing evidence. From X-rayed material of annual teosinte he obtained a tetraploid that is practically indistinguishable from perennial teosinte. The F_1 of crosses between perennial teosinte and maize are then in the nature of triploids, although it is certain that the maize and teosinte homologues are not exact duplicates (*1*), and it seems not improbable that there has been some differentiation of the duplicated chromosomes in *Euchlaena perennis*.

The somatic cells of an F_1 triploid plant have 3 more or less homologous chromosomes corresponding to each of the 10 haploid chromosomes of maize. At meiosis the normal procedure is for 2 of the 3 chromosomes to synapse and separate into different cells, the third member going at random to one or the other of these two cells.

Information regarding the similarity and differences of the duplicate chromosomes in perennial teosinte and the corresponding homologue in maize may be gained from the mating of chromosomes in the formation of gametes when all three homologues are present, i. e., the two teosinte chromosomes may pair with each other or 1 of them may pair with the maize homologue.

If the duplicate chromosomes in the perennial teosinte were exact homologues and differed from those of maize the two *Euchlaena perennis* chromosomes would be expected always to pair, leaving the

[1] Received for publication Oct. 10, 1934; issued April 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 133.

maize homologue to be distributed at random. On the other hand, if all three homologues were similar or had equal synaptic attraction, random pairing would be expected.

Present knowledge of the morphological characters of the chromosomes of perennial teosinte is too limited to identify the synaptic mates cytologically, but an indirect method of determining the nature of the pairings in F_1 hybrids is afforded by introducing with maize the waxy gene of the ninth chromosome and then observing the ratios of pure recessive, pure dominant, and heterozygous gametes derived from such a cross.

The gene for waxy endosperm (*wx*) is especially serviceable for measuring synaptic attractions because this gene is expressed in the pollen, thus making it possible to determine with any desired degree of accuracy the percentage of recessive male gametes. The procedure was to collect anthers about 1 day before pollen was shed, crush the pollen into a solution of iodine, and classify approximately 500 grains.

In the usual crosses of perennial teosinte and maize which produce a triploid F_1 the situation is complicated by the partial elimination of gametes with aneuploid chromosome numbers (*7*). In the pollen of a triploid F_1 there are many sterile grains, and these have a low number of chromosomes, thus reducing the percentage of waxy grains in the surviving pollen.

The fortunate occurrence of the tetrapoloid F_1 plant in which there were two sets of maize chromosomes and little or no sterile pollen affords an opportunity to determine the synaptic attractions of chromosome 9 in a very simple manner.

MEASURES OF AUTOSYNDESIS [3]

The two homologues of chromosome 9 contributed by the *Euchlaena perennis* parent may be designated X_1 and X_2, and the two from maize x_3 and x_4.

At meiosis the four homologues of chromosome 9 may pair in three ways: (1) X_1 with X_2, and x_3 with x_4; (2) X_1 with x_3, and X_2 with x_4; (3) X_1 with x_4, and X_2 with x_3. The first is autosyndetic and the second and third are allosyndetic. Since paired chromosomes pass to different gametes the four kinds of gametes formed following the first way of pairing will be $X_1 x_3$, $X_2 x_4$, $X_1 x_4$, and $X_2 x_3$, all genetically similar; i. e., there will be four Xx gametes. The gametes following the second way of pairing will be $X_1 X_2$, $x_3 x_4$, $X_1 x_4$, and $X_2 x_3$, and those from the third will be $X_1 X_2$, $x_3 x_4$, $X_1 x_3$, and $X_2 x_4$. The gametes following the second and third ways of pairing are genetically similar and the combined result of the allosyndetic pairings will be 2 XX, 2 xx, and 4 Xx. Thus, one-fourth of the gametes following allosyndesis will be recessive, whereas all of those following autosyndesis will contain a dominant gene. With equal synaptic attraction—i. e., random pairing—two-thirds of all the gametes will have followed allosyndesis and one-third autosyndesis. Accordingly, one-fourth of two-thirds, or one-sixth of the total gametes will be recessive.

With an increase in autosyndesis the percentage of recessive gametes decreases until with complete autosyndesis there will be no recessive

[3] Both the terms "autosyndesis" and "autosynapsis" have been used to designate synapsis inter se of like chromosomes. It would seem that the older term "synapsis" might have served in the new connection and syndesis is used in this paper not because it is thought more appropriate but because it seems to conform with current usage. The term "syndesis" was proposed by Häcker (*5*, *6*).

gametes. With an increase of allosyndetic pairings the percentage of recessive gametes increases until with complete allosyndesis 25 percent of the gametes will be recessive. Where there is no differential elimination of gametes, therefore, the intensity of autosyndetic attraction is indicated by the extent to which the percentage of recessive gametes departs from the 16.7 percent of random pairing.

The percentage of recessive gametes, however, is unsatisfactory as a means of comparing syndetic attractions. The distribution of percentages above and below the 16.7 is not symmetrical, and the increase of autosyndetic pairing indicated by a given minus departure from 16.7 percent does not equal the increase in allosyndetic pairings indicated by a plus departure of the same amount. As an example, if autosyndesis is increased from random pairing to such an extent that the resulting autosyndetic gametes are doubled in number, the percentage of recessive gametes will be reduced from 16.7 to 12.5, a difference of 4.2 percent. If, however, allosyndesis is doubled, the recessive gametes will be 20 percent, a difference of only 3.3 percent.

This skew distribution of percentages may be transformed to a scale running from -1 to 1, in which -1=complete allosyndesis, 0=random pairing, and 1=complete autosyndesis. Values on this scale may be termed the "coefficient of autosyndesis", and equal values above and below zero will represent equal percentage changes in allosyndetic and autosyndetic pairings. This coefficient of autosyndesis, which will be called t, is derived from the ratio of recessive gametes to all gametes, as follows:

$$t=\frac{1-6x}{1-2x}$$

where x= the ratio of recessive gametes.[4]

Since x is the only variable, the significance of t should equal the significance of the departure of x from 16.7 percent or

$$\sigma_t=\frac{t\sigma_x}{16.7-x}.$$

DESCRIPTION OF TETRAPLOID HYBRID

The F_1 hybrid that was found to have 40 chromosomes attracted attention while growing in the field because it was more cornlike than its sister F_1 plants. It had a major culm, and the minor culms were much reduced in size. The usual triploid F_1 shows very little differentiation of a major culm, and the few to many culms that develop the first year are of approximately equal size. Almost all the ears of the 40-chromosome F_1 had 2 rows of double alicoles, an occasional terminal ear having 4 rows of double alicoles. Normal F_1 hybrids usually have ears with 2 rows of single alicoles and only occasionally an ear

[4] If there is no elimination of aneuploid gametes this formula is applicable to triploid as well as to tetraploid hybrids. Where the mean chromosome number of the gametes is other than one-half the somatic number of the parent the value of t still may be determined if the mean number of chromosomes in effective gametes is known, as follows:

Let $x=\frac{\text{percent waxy pollen}}{100}$,

C=somatic number of chromosomes of parent plant,
c=mean number of chromosomes in the gametes,

$r=\frac{C-(c+10)}{C-20}$;

then $t=\frac{3x-r}{x-r}$.

with 4 rows. The 40-chromosome plant showed only 2 percent poorly developed pollen, while normal F_1 plants often have as high as 50 percent defective pollen. The percentage of *wx* pollen was between 4 and 5, a percentage higher than any thus far found in F_1 hybrids between perennial teosinte and waxy maize.

The fact that the F_1 plant had 40 instead of the usual 30 chromosomes, together with the habit of the plant and the more cornlike ears, suggested that it had 2 rather than 1 set of maize chromosomes in its complement. The various phases of the reduction divisions tended to confirm this view, since there was little or no indication of unpaired chromosomes and the distribution of the chromosomes to the daughter cells took place with only infrequent indications of irregularities.

The most natural explanation of the presence of the 2 sets of maize chromosomes is that the maize parent contributed 2 sets of chromosomes or that 1 maize set had doubled after fertilization. These are the explanations suggested by Emerson and Beadle (*3*) in describing another tetraploid hybrid between perennial teosinte and maize. The description of their plant indicates that in general morphological characters it was very similar to the tetraploid hybrids under discussion.

Chromosome studies of F_2 and F_3 generations continue to show very few irregularities in the reduction phases. In some plants the chromosomes on the first metaphase plates show many tetravalent chromosome groups; whether this is due to the association of 4 teosinte or 4 maize chromosomes or simply to the association of both teosinte and maize chromosomes in a group of 4 is difficult to say, since in a certain proportion of the plants of later generations the 4 homologues will be either all teosinte or all maize. The regular distribution of the chromosomes to the daughter nuclei is not disturbed by the presence of tetravalent groups; it is usual to find anaphase figures that have 20 chromosomes going to each pole, and consequently practically all pollen grains are euploid with 20 chromosomes.

PROGENY OF TETRAPLOID HYBRID (F_1)

The plant with 40 chromosomes produced pollen having 4.6 ± 0.94 [5] percent of the grains waxy, giving a coefficient of autosyndesis of 0.80 ± 0.06.

A plant with the composition *Wx Wx wx wx* would produce gametes with 0, 1, and 2 *Wx* genes, and when selfed there should be F_2 individuals with 0, 1, 2, 3, and 4 *Wx* genes.

If pollen and ovules behave alike the percentage of waxy pollen should make possible a prediction as to relative frequency of F_2 plants with 0, 1, 2, 3, and 4 *Wx* genes. Where $x = \frac{\text{percentage of waxy pollen}}{100}$ the expected frequencies would be:

Number of Wx genes	*Expected ratio*	*Plant characteristics*
0	x^2	Waxy seed
1	$2x\ (1-2x)$	50 percent waxy pollen
2	$2x^2+(1-2x)^2$	0–25 percent waxy pollen
3	$2x(1-2x)$	?
4	x^2	No waxy pollen

[5] Errors indicated by a ± sign are standard, not probable, errors.

Classification should be definite except for the class with three *Wx* genes. In the absence of irregular divisions, plants of this type should have no waxy pollen. Unequal divisions do occur, however, and their observed frequency indicates that about 0.5 percent of the gametes of a *Wx Wx Wx wx* plant would be waxy. Accepting this ratio, the chance of any particular pollen grain being waxy is 0.005, and in samples of 500 grains 92 percent of the samples would have one or more waxy grains and would fall in the low-waxy group. With regard to the upper limit, assuming the same probability of 0.005, about 90 percent of the samples from *Wx Wx Wx wx* plants would have less than 5 waxy grains, and would be listed as having less than 1 percent waxy pollen. For purposes of comparison, therefore, plants with less than 1 percent waxy pollen have been classed as *Wx Wx Wx wx*. There was no evidence of bimodality either in the F_2 or F_3 progenies, but in F_3 progenies from selfed *Wx wx wx wx* F_2 plants where there was no opportunity to produce *Wx Wx Wx wx* plants the lowest percentage was 4.

The tetraploid F_1 plant was self-pollinated and produced 276 seeds, 1 of which was waxy. The expected number of waxy seeds was 0.6.

DISTRIBUTION OF F_2 PLANTS

Fifty-six F_2 plants were grown, and approximately 500 pollen grains of each were classified by staining with iodine. The results are given under the column heading F_2 of table 1.

The 5 plants in the group approximating 50 percent waxy pollen are sharply differentiated from those with smaller percentages and undoubtedly are plants with one *Wx* gene. The one plant with no waxy pollen showed by the subsequent behavior of its progeny that it was homozygous *Wx*. The only uncertainty regarding the genetic composition of the plants is that of separating those with three *Wx* genes from those with two. The number of plants in the several groups is in good agreement with the expected.

DISTRIBUTION OF F_3 PLANTS

Nine F_3 progenies were grown in 1933 from nonwaxy seed of F_2 plants. Two progenies were from selfed plants with 50 percent waxy pollen (*Wx wx wx wx*), 4 were from selfed plants with a low percentage of waxy pollen (*Wx Wx wx wx*), and 3 were from crosses between the two groups (*Wx Wx wx wx*$\times$*Wx wx wx wx*).

A total of 435 F_3 plants were grown and the pollen grains of each were classified. With one exception the plants, like those of the F_2, fell into two well-defined groups with respect to percentage of waxy pollen. The single exception was a plant with 31.4 percent of waxy pollen in progeny C_3. This progeny was the result of selfing an F_2 plant with 51.3 percent of waxy pollen. The aberrant plant had 40 chromosomes and there was no indication of cytological irregularities. Until the descendants of this plant are grown no explanation can be offered for its unexpected percentage of waxy pollen; the plant is omitted from the tabulations.

The results are given in table 1 under the column heading F_3.

TABLE 1.—*Distribution of percentage of waxy pollen in F_2 and F_3 plants*

Genetic group and percentage of waxy pollen (class value)	F_3 plants of indicated parentage									F_2 plants
	Progenies (upper) and percentages of waxy pollen (lower) of *Wx wx wx*×self				Progenies (upper) and percentages of waxy pollen (lower) of *Wx wx wx wx*×self		Progenies (upper) and percentages of waxy pollen (lower) of *Wx wx wx wx* × *Wx Wx wx wx*			
	C_2 7.9	C_6 11.2	C_7 4.0	C_9 1.5	C_1 50.9	C_3 51.3	C_4 51.3×7.9	C_8 4.0×50.9	C_{10} 1.5×50.9	
Wx Wx Wx Wx Wx:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
0	0	0	0	0	0	0	0	0	0	1
Wx Wx Wx wx:										
0.1–0.9	3	1	4	2	0	0	1	0	2	2
Wx Wx wx wx:										
1.0–1.9	5	2	8	1					1	4
2.0–2.9	6	3	14	7				1	2	8
3.0–3.9	15	5	15	9				1	3	7
4.0–4.9	9	5	12	13	3			4	1	8
5.0–5.9	0	6	7	6	1	4	3	2	2	6
6.0–6.9	1	3	4	4	2	2	3	4	2	2
7.0–7.9	2	6	1	0	2	1	1	2	0	5
8.0–8.9	1	5	1	1	1	3	4		4	2
9.0–9.9	4	3	1	2	1	0	3		3	3
10.0–10.9	3	4	0		0	1	0		1	1
11.0–11.9		4	1		0	1	0		1	1
12.0–12.9		0			1	0	4	1		
13.0–13.9		0			1	0	3			
14.0–14.9		1				0				
15.0–15.9		1				1				
16.0–16.9		1								1
Plants, number	46	49	64	43	12	13	21	15	20	48
Mean waxy pollen, percent	4.7	7.2	4.0	4.5	7.6	8.1	9.4	5.9	6.5	5.2
Observed σ	2.7	3.5	2.0	1.8	3.0	2.8	2.9	2.3	3.0	3.1
σ_{50}	11.1	13.7	10.3	10.8	14.3	14.8	16.1	12.5	13.2	11.7
Expected σ	.95	1.16	.88	.93	1.19	1.22	1.31	1.06	1.10	.99
Wx wx wx wx:										
44.0								1		
45.0								0	1	
46.0								0	1	
47.0						4		2	1	
48.0						4	2	0	0	
49.0			1	2	3	8	4	3	1	
50.0		4	0	0	9	7	5	1	2	2
51.0		0	0	3	3	6	2	2	1	1
52.0		2	1	1	2	5	5	3		1
53.0		0	0	1	3	7	0	1		1
54.0		0	1		2	1	1	1		
55.0		1	1		2	1	1	1		
56.0		0			2					
57.0		1					1			
58.0										
59.0										
60.0							1			
Plants, number	0	8	4	7	26	43	28	15	7	5
Mean waxy pollen, percent		52.5	53.0	51.4	52.2	50.9	52.2	5.08	48.8	51.7
Observed σ		2.7	2.6	1.5	2.2	2.1	2.7	2.9	2.3	1.3
Expected σ		2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2

In some progenies the number of plants in the several genetic groups depart rather widely from the expected, but the totals are in fair agreement.

The number of plants classified as having three *Wx* genes is low, which may indicate that some of the plants with more than 1 percent of waxy pollen should have been placed in this group.

PERCENTAGE OF WAXY POLLEN IN F_2 AND F_3

The percentage of waxy pollen in individual plants having the composition *Wx wx wx wx* was a close approximation to the expected 50 percent. The progeny means, ranging from 48.8 to 53.0 percent in some instances, deviated significantly from 50 percent, but the departures are no greater than are commonly observed in maize hybrids. Plants having the composition *Wx Wx wx wx* ranged from 1.1 to 16.6 percent waxy pollen. Progeny means ranged from 4.0 to 9.4 percent, many of the differences being of undoubted significance. The values are given in table 1.

COEFFICIENT OF AUTOSYNDESIS

The percentage of waxy pollen of any plant with two *Wx* genes affords a measure of autosyndesis in that plant. Of this type there were, in addition to the F_1 individual, 48 F_2 and 283 F_3 plants. In the F_1 the coefficient of autosyndesis $t=0.80$. The mean value of t in the F_2 was 0.77 ± 0.03. In the entire population of F_3 progenies the mean value of t was 0.74 ± 0.05. The slight evidence of a decrease in t is insignificant.

The 283 F_3 plants were in 9 progenies. Two of these progenies were the result of selfing F_2 plants with approximately 50 percent waxy pollen, 4 resulted from selfing F_2 plants with a low percentage of waxy pollen, and 3 were from crosses between plants with a low percentage of waxy pollen and plants with approximately 50 percent waxy pollen.

The mean values of t in these three groups were as follows:

Parents	t	*Standard error*
50 percent×self	0.63	0.05
Low percent×self	.78	.05
50 percent×low	.65	.08

Again the differences are insignificant, the differences between the extremes being only 2.16 times the standard error of the difference. It should perhaps be noted that the order of the coefficients is that expected if crossing-over, which may have occurred in the F_1 and F_2, were a factor in reducing autosyndesis.

VARIATION IN COEFFICIENT OF AUTOSYNDESIS

One of the most striking things brought out in table 1 is the greater variability of the percentage of waxy pollen in plants with two *Wx* genes than in plants with one *Wx*. The magnitude of this difference is not apparent in the standard deviations of the arrays, the true difference being obscured by the fact that the expected random variation due to errors of sampling decreases as the mean ratio departs from 0.5 or 50 percent. The standard deviations may be made comparable by multiplying each standard deviation by the factor $\frac{0.5}{\sqrt{pq}}$, where $p=\frac{\text{percent waxy}}{100}$ and $q=1-p$. Standard deviations thus corrected to a base of 50 percent are designated σ_{50} in table 1. For the plants with one *Wx* the mean values are so close to 50 percent that the correction is negligible. A comparison of these corrected values shows the standard deviation of the plants with two *Wx* genes

to be from 4 to 9 times that of plants in the same progeny with one *Wx*.

The same difference is apparent if the observed standard deviations are compared with their respective errors. In every progeny the standard deviation of plants with one *Wx* was a close approximation to the 2.2 percent expected as a result of sampling errors in samples of 500. In the plants with two *Wx* genes the observed standard deviation is significantly greater than the expected in every progeny.

A general comparison of the variability of plants having one *Wx* gene with the variability of those having two *Wx* genes can be made by analyzing the total variance of the entire population of F_3 plants. Snedecor (*9*) describes the analysis of variance as "a technique for segregating from comparable groups of data the variation traceable to specified sources."

As applied to the present data the analysis of variance combines all the classified pollen grains from F_3 plants in each of the genetic groups into a single population and segregates the total variance into the portions contributed by the several subdivisions. The total variance is $\overline{pq}\ \overline{N}$, i. e., the $\frac{\text{percent waxy}}{100}$ times the $\frac{\text{percent horny}}{100}$ of the entire population times the total number of pollen grains. A portion of this total variance will be accounted for by the $pq\ N$ of the individual plants, another portion will be due to departures of the percentage of waxy pollen of individual plants from the mean of the progeny in which they occur, still another portion will be due to departure of the progeny means from the mean of the type of cross—i. e., 1 *Wx* by self, 2 *Wx* by self, and 1 *Wx* by 2 *Wx*—and lastly there will be a portion due to departures of means of the three types of crosses from the general mean. If these several divisions constituted merely random samples from a homogeneous population, the mean square would be essentially the same for all the divisions. The values of mean-square differences required for significance are given in a table at the end of Snedecor's work (*9*) and by Fisher (*4*).

All sums of squares have been divided by 500, which, without changing the relative values, reduces the mean squares to the basis of the variance of means of plants in the progeny.

The analysis of variance of all the F_3 plants of the composition *Wx wx wx wx* and *Wx Wx wx wx* is shown in table 2.

TABLE 2.—*Analysis of variance of all F_3 plants of the composition Wx wx wx wx and Wx Wx wx wx*

Genetic composition and source of variation	Degrees of freedom	Sum of squares	Mean square
Wx wx wx wx:			
Types of parental cross	2	15	7.5
Progenies in type	5	135	27.0
Plants in progeny	131	729	5.6
Pollen in plant	69,361	346,457	5.0
Wx Wx wx wx:			
Among means of types of parental cross	2	366	183
Progenies in type	6	452	75
Plants in progeny	274	1,937	7.1
Pollen in plant	141,217	138,311	.98

In the plants with one *Wx* the mean-square values are practically alike except the value for the means of progenies. This value is significantly larger than the mean square of plants in the progeny or of pollen in individual plants. The probability is somewhat less than 0.01 in both cases.

In plants with two *Wx* genes the variation among types of the parental cross is the only class that does not show a significant difference in comparison with other sources of variation.

The point of interest in connection with variation in autosyndesis is that in the plants with one *Wx* gene (where there is no opportunity for autosyndetic attractions to operate) variation of plants from the mean of the progeny is no greater than that expected from sampling errors, whereas in the plants with two *Wx* genes the variance of plants from the mean of the progeny is over seven times the expected.

The variation in the percentage of waxy pollen might result from a differential survival of the waxy pollen before the pollen was classified, but if so the same differentiation would be expected in the plants with one *Wx* gene and it seems more reasonable to associate the variation with variation in autosyndesis.

There is evidence that the tendency toward autosyndesis is inherited. The four F_3 progenies of the 2-*Wx*-by-self type of cross have a parent-offspring correlation of 0.82 between the mean percentage of waxy pollen of the parent plants and the mean percentage of the progenies. As this value is based on but four pairs it is not significant, but since the values for the offspring are the means of 50 or more plants the standard error must be somewhat less than the indicated value of 0.18.

SEED CLASSES

The F_3 plants that provided the material for estimating the ratio of autosyndesis to allosyndesis in the formation of pollen were pollinated with pollen from pure waxy plants. The seed thus produced provided material for comparing the behavior of the ovules with that of the pollen. The number of seeds obtained from individual plants was too small—few plants produced more than 100 seeds—to justify the kind of analysis used with the pollen. The numbers are sufficient however, to supplement some of the conclusions based on the pollen counts. About 270 F_3 plants of which the pollen was counted produced seed. In no plant was there a disagreement regarding the indicated genetic constitution. Classified on the percentage of waxy seed the plants fell into two groups, one approximating 50 percent and the other having a low percentage; these groups corresponded to those based on the pollen classification.

Rather critical evidence that the variation in the percentage of waxy in the low-percentage group is caused by variation in autosyndesis is provided by the correlations between the percentage of waxy pollen and the percentage of waxy ovules of the same plant. If variation in the percentage of waxy is the result of differences in the coefficient of autosyndesis, variations in syndetic attraction might be expected to affect pollen and ovules alike and there should be a positive correlation between the percentage of waxy pollen and the percentage of waxy ovules in the low-percentage group. In the 50-percent group, where one-half of all pairings must be between mates and

one-half between nonmates, the variations in percentage of waxy are assumed to be due to sampling errors and there should be no correlation between pollen and ovules. In 64 plants in the low-percentage group with a total of 7,106 seeds, the total correlation was 0.36; that between the means of the 8 progenies, 0.72; and that within the progenies, 0.27. The total correlation is undoubtedly significant ($P=0.0016$). The interprogeny and intraprogeny coefficients have each a probability of 0.02. Although the numbers are small the interprogeny correlation indicates that the tendency to autosyndesis is inherited.

In the 50-percent group there were 93 plants with 8,228 seeds. The correlation coefficients (total, -0.065; between means, 0.015; within progenies, -0.071) were as near zero as could be expected.

SUMMARY

The occurrence of a tetraploid plant among F_1 hybrids between perennial teosinte (*Euchlaena perennis* Hitchc.) and waxy maize (*Zea mays* L.) is reported.

The tetraploid plant was more maizelike in its morphological characters than the usual triploid hybrids of the same parentage. The 40 chromosomes of the somatic tissue of the tetraploid plant are believed to be made up of 20 from the perennial teosinte, the normal haploid number, and 2 sets of maize chromosomes.

Meiosis was regular and there were very few sterile pollen grains.

At meiosis the pairing may be of two types: (1) The teosinte chromosomes may pair with each other and the maize chromosomes do likewise, which is autosyndesis, or (2) the teosinte and maize homologues may pair, which is allosyndesis. The relative frequency of these two types of pairing may be measured by the percentage of gametes carrying the recessive gene *wx* introduced with the maize parent.

A formula is given for calculating the coefficient of autosyndesis, which transforms the skew distribution of percentages into a symmetrical distribution. With this measure, called t, complete autosyndesis is represented by 1, random pairing by 0, and complete allosyndesis by -1.

The F_2 and F_3 progenies produced plants with 0, 1, 2, 3, or 4 dominant *Wx* genes in approximately the expected ratios, indicating a similarity in the behavior of pollen and ovules.

In the F_1 plant $t=0.80$. The mean value of t in F_2 was 0.77. Nine F_3 progenies showed means ranging from 0.54 to 0.82.

The percentage of waxy pollen in F_2 and F_3 plants with two dominant *Wx* genes was much more variable than in plants with one *Wx*.

The greater variability in the percentage of waxy pollen grains in plants with two *Wx* genes as compared with those with one *Wx* is ascribed to individual variations in autosyndetic attraction.

In plants with one *Wx*, where there was no possibility of variation in the ratio of autosyndetic pairings, the observed intraprogeny variability of percentage of waxy pollen could all be attributed to sampling errors. In plants with two *Wx* genes, where variations in autosysdesis would change the percentage of waxy pollen, the variation could not be ascribed to chance.

Correlations between the percentage of waxy pollen and the percentage of waxy ovules gave supporting evidence of variation in the coefficient of autosyndesis. In the plants with one *Wx* gene there was no correlation, while in the plants with two *Wx* genes the correlation was significant.

There is fairly good evidence that the variations in autosyndesis are inherited.

LITERATURE CITED

(1) BEADLE, G. W.
1932. STUDIES OF EUCHLAENA AND ITS HYBRIDS WITH ZEA. Ztschr. Induktive Abstamm. u. Vererebungslehre 62: 291–304, illus.

(2) EMERSON, R. A.
1929. GENETIC NOTES ON HYBRIDS OF PERENNIAL TEOSINTE AND MAIZE. Amer. Nat. 63: 289–300.

(3) ——— and BEADLE, G. W.
1930. A FERTILE TETRAPLOID HYBRID BETWEEN EUCHLAENA PERENNIS AND ZEA MAYS. Amer. Nat. 64: 190–192, illus.

(4) FISHER, R. A.
1932. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, rev. and enl., 307 pp., illus. Edinburgh and London.

(5) HÄCKER, V.
1904. BASTARDIRUNG UND GESCHLECHTSZELLENBILDUNG. Zool. Jahrb. Sup. 7; [161]–256, illus.

(6) ———
1907. DIE CHROMOSOMEN ALS ANGENOMMENE VERERBUNGSTRÄGER. Ergeb. u. Fortschritt. Zool. 1, 72 pp.

(7) LONGLEY, A. E.
1934. CHROMOSOMES IN HYBRIDS BETWEEN EUCHLAENA PERENNIS AND ZEA MAYS. Jour. Agr. Research 48: 789–806, illus.

(8) RANDOLPH, L. F.
1931. X-RAYED SEED OF ANNUAL PLANT PRODUCES PERENNIAL. U. S. Dept. Agr. Press Release, September 20, 1931.

(9) SNEDECOR, G. W.
1934. CALCULATION AND INTERPRETATION OF ANALYSIS OF VARIANCE AND COVARIANCE. 96 pp. Ames, Iowa. (Iowa State Col., Div. Indus. Sci. Monog. 1).

DICTYOSTELIUM DISCOIDEUM, A NEW SPECIES OF SLIME MOLD FROM DECAYING FOREST LEAVES[1]

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INTRODUCTION

In the course of studies made in this laboratory on the amoeboid population of soil and decaying vegetable matter, myxamoebae in large numbers have been regularly encountered. Thom and Raper (*8*)[3] reported that the amoeboid stage of some Myxomycetes formed a normal part of the microbiological population of field soil and decomposing crop residues, and could readily be isolated and grown in artificial culture. Following this, Raper and Thom (*6*) studied the distribution of the Acrasieae in soil and reported *Dictyostelium* as a common component of the soil population from widely scattered areas and from many types of soil, while *Polysphondylium* was repeatedly isolated from vegetable remains, particularly forest litter, and occasionally from soil.

These results were in close agreement with earlier work by Krzemieniewski (*3*) and extended her observations in this field. It was reported previously (*6*) that the vast majority of cultures of *Dictyostelium* isolated apparently belonged to the single species *D. mucoroides* Bref. Continued studies and isolations since that time have shown an equally large proportion belonging to that species and support the writer's earlier findings. However, not all cultures isolated could be identified with *D. mucoroides*. A form closely agreeing with the description of *D. sphaerocephalum* (Oud.) Sacc. and March., as given by Olive (*4*) in his comprehensive study of the group, has been occasionally obtained from decaying forest litter. *D. purpureum* Olive has been isolated once, from decaying sphagnum. Still another form has been isolated that does not agree with the description of any published species and differs fundamentally in some respects from the other members of the genus. It has seemed desirable, therefore, to describe it as a new species, to review briefly its life cycle, and to discuss at some length the formation and behavior of certain structures not seen in other species.

TECHNICAL DESCRIPTION

Dictyostelium discoideum, n. sp.

Soris griseo-albis vel citrinis, rotundatis, apiculatis, plerumque 125μ–300μ diam.; sorophoris griseo-albis, ex discis expansis oriundis, basi rigidis, ad apicem tenuibus, flexuosis attenuatis, 1.5–3 mm altis; discis basilaribus cellularibus, conicis, bases sorophororum circumvallentibus et sustentantibus, 150μ–400μ diam.; sporis anguste ellipticis, hyalinis, 6μ–9μ × 2.5μ–3.5μ.

[1] Received for publication Nov. 19, 1934; issued April 1935.

[2] The writer is indebted to Charles Thom, Division of Soil Microbiology, for valuable counsel and criticism regarding the work, and to Edith K. Cash, Division of Mycology and Disease Survey, for preparing the Latin diagnosis.

[3] Reference is made by number (italic) to Literature Cited, p. 147.

Hab. in foliis putrescentibus in silva decidua, North Carolina.

Colonies grown on hay and dung agar; sori grayish white to pale lemon yellow, rounded, apiculate, commonly 125μ–300μ in diameter, with larger and smaller specimens frequent; sorophores grayish white, arising from expanded disklike bases, upright, rigid below, 30μ–80μ in diameter, tapering to thin flexuous above, 5μ–15μ in diameter, 1.5–3 mm high, less commonly longer or shorter; basal disks cellular, conical, surrounding and supporting bases of sorophores, 150μ–400μ in diameter; spores narrowly elliptical, hyaline, 6μ–9μ by 2.5μ–3.5μ.

Isolated from decaying leaves from deciduous forest, North Carolina.

One of the most striking characters of this species, which is only suggested in the mature sorocarp [4] by a trail of slime leading away from the base, is the peculiar behavior of the pseudoplasmodium. In other described species of *Dictyostelium*, three of which, *D. mucoroides*, *D. sphaerocephalum*, and *D. purpureum*, the writer has studied in culture, the fruiting stalk is produced from the point where the myxamoebae congregate. In this species the myxamoebae come together to form an aggregate, or pseudoplasmodium, as in the other and more common forms, but in the ordinary laboratory culture, instead of developing into a sorocarp immediately, the myxamoebae compact themselves together to form an elongated cylindrical mass which moves as a unit across the agar plate for a greater or less distance before pausing to complete its cycle of development. The formation, structure, and behavior of this "migration pseudoplasmodium" will be considered in greater detail later.

ISOLATION AND CULTURE

Dictyostelium discoideum was isolated from decaying leaves collected in a hardwood forest of the North Carolina mountains in the summer of 1933. The dominant trees were beech, birch, oak, and buckeye, and the sample consisted largely of the partially decomposed leaves of these trees, together with some weed residues. The sample had a pH of 4.65.

In isolating the organism, culture methods similar to those reported by Raper and Thom (*6*) were used. The sample was ground in a clean mortar with approximately 5 parts of sterile water, and the resulting suspension was streaked upon mannite agar plates. The plates were incubated for 3 weeks at 18° to 20° C. At the end of this time spores from sori uncontaminated by fungi were transferred to

[4] Following Zopf's use of the terms "sorus" for the spore mass and "sorophore" for the supporting structure, or stalk, Harper (*2*) introduced the term "sorocarp" to include the whole fruiting structure.

EXPLANATORY LEGEND FOR PLATE 1

A.—Mature sorocarp photographed from the side, showing the typical lemon-shaped sorus, the erect, evenly tapered sorophore, and the expanded basal disk. × 15.

B.—Spores. × 900.

C.—Vegetative myxamoebae growing in a bacterial colony; killed and stained with rose bengale and photographed in situ on the culture plate. × 250.

D.—Vegetative myxamoebae stained as in *C* and photographed in higher magnification, showing bacterial cells in the surrounding medium. × 900.

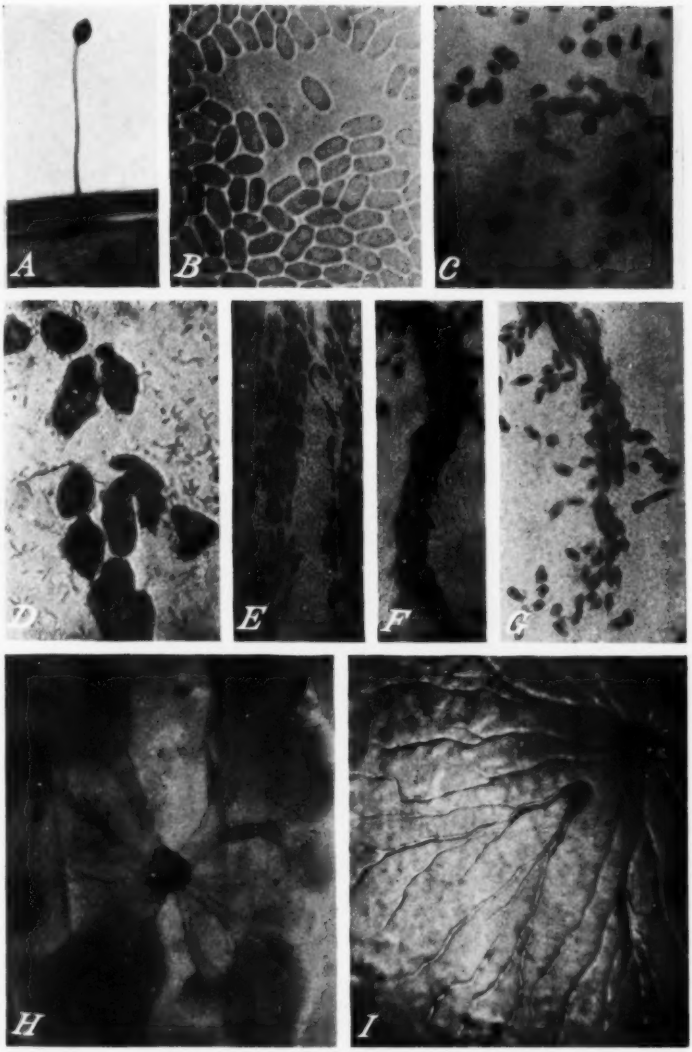
E.—Early stage in the orientation and aggregation of myxamoebae to form a pseudoplasmodial stream. In the lower part of the picture but little orientation of the myxamoebae is evident and aggregation is just beginning, while above a definite stream is already formed. × 250.

F.—A slightly later aggregation stage, but one in which the myxamoebae remain in a single layer. Their elongate Limax form and rather uniform orientation are shown. Movement is toward the top of the photograph. × 250.

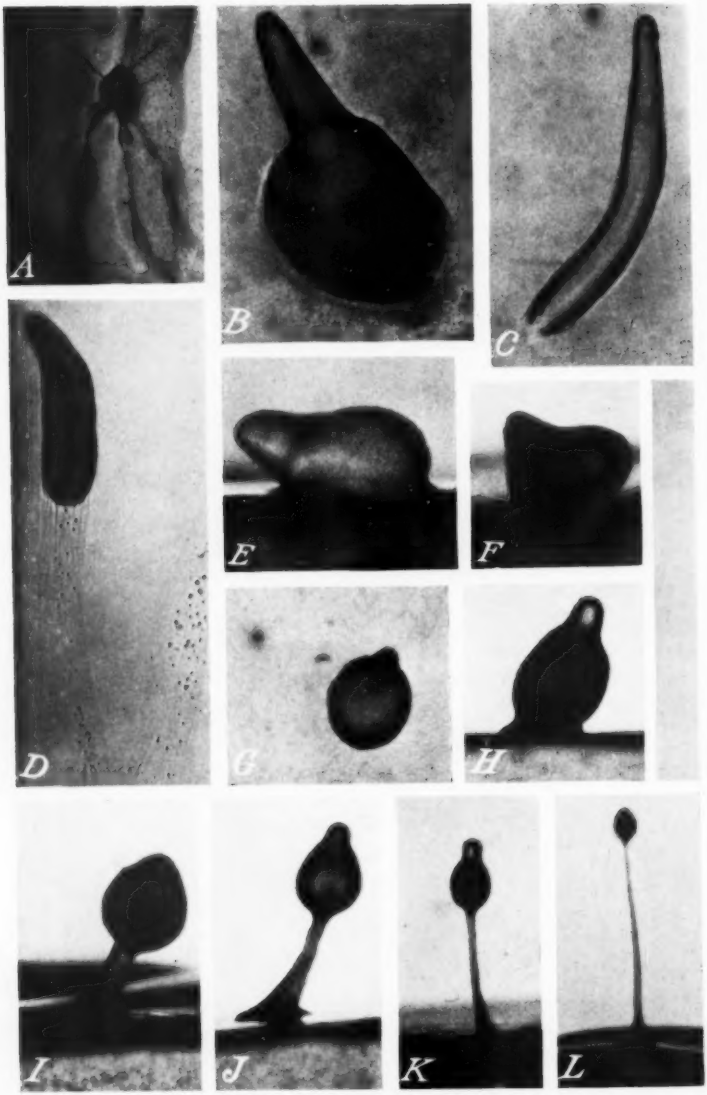
G.—A somewhat later stage in aggregation of the myxamoebae, which here have become compacted together to form a ropelike structure. × 250.

H.—Entire pseudoplasmodium with streams of myxamoebae radiating in all directions from the aggregation center shown as an irregular darkened area. Note that the streams can be traced directly to regions of heavy bacterial growth, which appear as darker areas in the photograph. × 15.

I.—Somewhat later stage of aggregation, showing a large pseudoplasmodium surrounding a smaller one. In an earlier stage the entire colony was flowing toward a common center, upper right; then for some reason a main stream was severed, and as a result two fruiting masses will develop. × 15.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

fresh plates of hay- and of dung-infusion agar adjusted to a pH of 6.0. The organism has been grown continuously upon these media and in Petri-dish cultures since that time. Transfers have been made at intervals of 2 to 3 weeks, although it is not necessary to transfer so frequently to maintain the stock culture. Cultures have been grown for the most part in a dark incubator at a temperature of 18° to 20°.

In photographing and in measuring the dimensions of myxamoebae and in studying their relation to one another in the pseudoplasmodium it is essential to disturb the organisms as little as possible. It is particularly difficult to obtain and to maintain a true picture of them as they naturally occur in culture. If they are transferred to slides they change shape and position during the process, and if they are observed on the agar plate the concentration of light and heat when high powers are used induces the same changes. To obviate these difficulties as far as possible, photographs and measurements of myxamoebae have been made of specimens killed and stained in situ with rose bengale[5]. This preparation kills the organisms immediately and stains them a darker red than the underlying medium, so that they stand out clearly and their actual form and position, as they occur in culture, are preserved. However, the stain has a decided disadvantage in that it stains the whole organism indiscriminately and does not permit cytological study. Only the figures shown in plate 1, *C* to *G*, are photographs of myxamoebae thus killed and stained in situ; other photographs are of unstained, living material. All the photographs were made with a Leitz Makam camera.

VEGETATIVE STAGE

When large numbers of spores (pl. 1, *B*) are planted upon fresh dung- or hay-infusion agar plates, germination begins within the first day and continues for 2 or 3 days thereafter. It is characterized by a swelling of the spore contents and a longitudinal splitting of the spore case, from which the protoplast emerges as a myxamoeba. The process is similar to that in *Dictyostelium mucoroides* and agrees closely with Olive's figures (*4, pl. 6, figs. 42, 44*).

In the vegetative stage following germination, the myxamoebae are quite hyaline and finely granular, and show little differentiation into endoplasmic and ectoplasmic regions. They are uninucleate and

[5] One-percent rose bengale, or erythrosin, in 5-percent solution, to which is added a trace of calcium chloride.

EXPLANATORY LEGEND FOR PLATE 2

A.—A pseudoplasmodium in approximately the same stage of development as that shown in plate 1, *I*, with streams of myxamoebae leading in from the bacterial streaks on either side of the aggregation center. × 15.

B.—A migrating pseudoplasmodium leaving a bacterial colony. × 50.

C.—Migrating pseudoplasmodium. The transverse line in the anterior end marks the point of contact with the agar surface. × 50.

D.—Migrating pseudoplasmodium with a slime streak marking its former path. Another slime streak cuts diagonally across the lower right-hand corner of the photograph. × 50.

E.—Side view of a pseudoplasmodium just before it ceases migration. × 50.

F.—Side view of a pseudoplasmodium in which movement has ceased and sorocarp formation is beginning. × 50.

G.—Similar stage of development as that shown in *E*, but viewed from above. × 50.

H.—Early stage in formation of the sorocarp. Mass of myxamoebae becoming divided into spore- and sorophore-forming regions. × 50.

I, J.—Successive stages, showing the sorogenic mass ascending the sorophore as it develops. × 50.

K.—Still later stage, showing a well-formed apical papilla in which active sorophore formation is taking place. × 30.

L.—Mature sorocarp. × 16.

regularly possess a single contractile vacuole, which occupies a posterior position in the cell. Food vacuoles and vacuoles surrounding foreign bodies are often clearly evident.

In shape the myxamoebae vary widely. They are not infrequently elongate or of irregular shape, owing to the presence of extended pseudopods, but are more commonly rounded or broadly triangular. Plate 1, *C* and *D*, illustrates clearly this condition. The photographs are of myxamoebae killed and stained with rose bengale and photographed in situ on the agar plate, and thus faithfully show them as they appear when actively vegetating. In hanging-drop cultures, the myxamoebae are more irregular in outline and frequently show few to many thin, finely pointed pseudopods, particularly in the anterior region. In size the vegetative myxamoebae vary from 12μ to 20μ by 8μ to 12μ, with the majority about 14μ to 16μ by 9μ to 11μ. Measurements in such amoeboids are subject to rapid change with variations in shape, however, and cannot be relied upon with the same confidence as measurements of spores and other definite walled structures. It is obvious that an amoeba, while remaining constant in volume, would show, when flattened and actively moving, entirely different dimensions from those it would show when rounded and relatively quiescent.

The present studies add little to the controversial subject of the nutrition of myxamoebae. The vegetative myxamoebae of *Dictyostelium discoideum* reach their maximum development in bacterial colonies, outside the limits of which they occur as scattered individuals only. The writer has not yet been able to determine satisfactorily whether the greater amount of moisture in the bacterial colonies induces the concentration of the myxamoebae there, or whether the myxamoebae feed upon the products of bacterial metabolism or utilize substances in the substratum set free by bacterial activity, or whether they feed directly upon the bacterial cells. It is not uncommon to see myxamoebae with vacuoles enclosing bacteria, but to determine whether the bacteria are undergoing digestion or are merely present as so much inert material, as Olive (*4*) has indicated, is quite another matter. Pinoy (*5*), working with *D. mucoroides* and *D. purpureum*, and later Skupienski (*7*), working with the former species only, have reported the presence of bacteria as essential to the growth of these species. Pinoy considered the myxamoebae as parasitizing the bacterial colonies, while Skupienski believed that a symbiotic relation exists. Just what the relation is between the two in *D. discoideum* the writer is not prepared to say; certainly the myxamoebae of this species are not hindered in any way by the presence of a fair growth of "contaminating" bacteria.

Under favorable culture conditions the vegetative stage, that is, the period from spore germination to the beginning of pseudoplasmodium formation, lasts several days, and during that time the myxamoebae increase tremendously in number. The first pseudoplasmodia appear in 3 to 4 days, and others continue to develop for a similar period. The two stages, vegetative and fructifying, are thus seen to proceed simultaneously for a time in the same culture. Cell division of the vegetating myxamoebae of this species has not been studied.

AGGREGATION STAGE

Following a vegetative period, the myxamoebae congregate toward definite centers and form aggregations preparatory to fruiting. The process here agrees so closely with that in other *Dictyostelia* to which Harper (*2*) and Olive (*4*) have given such detailed study that it is only necessary to discuss it briefly. The first evidence of aggregation is the appearance of certain areas of closely packed myxamoebae. The stimuli involved in this aggregation have never been determined, but these strategically placed individuals apparently exert some stimulus which reaches myxamoebae in the surrounding area and causes them to become elongated and oriented in the direction of these centers and to flow toward them. Gradually the orientation proceeds outward and the colony of aggregating myxamoebae takes the form of definite streams converging toward definite centers. Plate 1, *E* to *G*, shows different stages in this process.

The formation of a pseudoplasmodial stream is shown first by the orientation and aggregation of scattered myxamoebae (pl. 1, *E*). In the outer edges of the area involved they appear only slightly different from vegetative forms and show little orientation, whereas toward the center of the developing aggregate the orientation is much more pronounced, the myxamoebae are crowded together, and the pseudoplasmodial stream is an accomplished fact. Plate 1, *F*, shows a slightly later stage, in which the myxamoebae are definitely out of the vegetative condition and are actively moving toward the aggregation centers. However, they are not compacted together to any particular degree and still remain in a single layer on the agar surface. The degree of elongation of the aggregating myxamoebae is shown here. In higher magnifications they are clearly differentiated into endoplasmic and ectoplasmic areas, the latter occupying a broad band at the anterior end of the myxamoebae. As the stream becomes slightly older, the myxamoebae are interlocked and overlap to such a degree as to form a compact ropelike mass (pl. 1, *G*).

The three photographs (pl. 1, *E–G*), although not of the same stream nor even of the same aggregate, are fairly typical of successive stages in the orientation of the myxamoebae and the formation of the pseudoplasmodium, and at the same time fairly represent sections of the stream successively nearer the aggregation center. All three photographs were made near the tips of streams, as it was quite impossible to photograph the larger streams near the centers and show details of individual myxamoebae.

Plate 1, *H* and *I*, and plate 2, *A*, show a lower magnification of entire aggregations. A pseudoplasmodium with streams of myxamoebae radiating in all directions from the center, which appears as a darkened irregular area, is shown in plate 1, *H*. The streams can be traced directly to regions of heaviest bacterial growth, which appear as darker areas in the photograph. Plate 2, *A*, which was taken at about the same stage in development, shows this condition equally well. The myxamoebae are being drawn primarily from the two bacterial streaks shown on either side of the aggregation center. The two pseudoplasmodia shown in plate 1, *I*, represent a later stage of development, in which many small streams have merged to form larger streams which appear as definitely raised and ropelike struc-

tures. The streaming and aggregation of the myxamoebae continue until a compact, elongate cylindrical mass is formed.

In plate 1, *I*, is further shown a very large pseudoplasmodium surrounding, as it were, a much smaller one, and in this case it is obvious that in an earlier stage the whole colony of myxamoebae was flowing toward a common aggregation center. Then, from some cause either foreign to or within the pseudoplasmodium or its component cells, a new center appeared, and as a result two fruiting masses will develop, each producing a sorocarp. This may well raise the question of just what determines the size of an aggregation. As Van Tieghem (*9*), Olive (*4*), Harper (*2*), and others have shown, the myxamoebae that make up a pseudoplasmodium retain their individuality, are mechanically separable, and react more or less independently of one another; none the less it is equally apparent that there is some stimulus or force which influences and determines what myxamoebae shall go into one aggregation and what into another, and consequently how large the fruiting structures shall be. In an even culture of myxamoebae, what determines whether there shall be more and smaller or fewer and larger sorocarps? For the present the whole subject must remain a matter of conjecture.

The developing pseudoplasmodium may be of almost any conceivable shape. In a fresh culture with an even distribution of myxamoebae, the form is typically radially symmetrical. But in an older culture where previous aggregations have already withdrawn the myxamoebae from certain areas, or in fresh cultures where there is an uneven growth of amoeboids, the centers once formed draw the myxamoebae from wherever they can. A resulting pseudoplasmodium may consist of a single extended stream, or of two streams extending out in opposite directions, or may be triangular as myxamoebae are drawn from a sector uninfluenced by previously developing masses, or even crescent-shaped as it forms at the periphery of a bacterial colony.

MIGRATION STAGE

Thus far the development of *Dictyostelium discoideum* follows closely that of other species of the genus. But at this point a stage occurs which does not appear in any of the Acrasieae described. The myxamoebae come together to form pseudoplasmodia which, instead of initiating a sorophore formation at once, gradually become transformed into compact, elongated cylindrical masses by the continued streaming and crowding together of their component myxamoebae. Sorophore formation is delayed, and between the appearance of the compact cylindrical pseudoplasmodium, as described above, and the beginning of sorophore formation, a period ensues during which the pseudoplasmodium moves as a unit over the surface of the agar until it reaches a suitable location for sorocarp formation. As shown earlier in this paper, the myxamoebae grow most luxuriantly in bacterial colonies, and the pseudoplasmodia are regularly formed within the limits of these colonies; thus the migratory stage may well be a demonstration of negative hydrotaxis, that is, a concerted movement on the part of the pseudoplasmodium to reach a drier situation in which to develop the fruiting structure. Plate 2, *B*, shows such a pseudoplasmodium leaving a bacterial colony.

In shape the typical migrating pseudoplasmodium is elongate cylindrical, with the anterior end tapering to a greater or less degree and the posterior end rather blunt and somewhat flattened horizontally (pl. 2, *C*). However, variations from this form are not uncommon; the moving mass may be longer and narrower, with a less pointed tip, or shorter and relatively thick, with the anterior end rounded rather than pointed. It may be either straight or slightly curved (pl. 2, *C*). As it moves, the greater portion of the mass is regularly in intimate contact with the agar surface, but with the pointed frontal portion slightly raised above it; the transverse line in the upper half of the pseudoplasmodium (pl. 2, *C*) marks the point where contact with the agar surface is interrupted. In long narrow specimens the mass may be raised in the central portion as well.

In size the migrating pseudoplasmodia vary widely, both as to relative dimensions of length and breadth, as pointed out above, and as to total volume. But in well-developed cultures on hay-infusion agar, which have been incubated for 5 or 6 days at 18° C., typical specimens show dimensions of about 0.8 to 1.2 mm by 0.15 to 0.25 mm, with extremes measuring as much as 2.0 mm in length and as little as 0.4 to 0.5 mm by 0.07 to 0.1 mm. With some exceptions, volume is clearly dependent upon the extent of the aggregating mass from which the migratory structure developed, but the factors which determine the relative proportion of length to breadth are not so obvious.

If a migrating pseudoplasmodium is crushed in a drop of water under a cover slip, the entire mass is seen to be composed of apparently similar and undifferentiated cells. There is not the slightest indication of a stalklike structure, and the myxamoebae readily and quickly separate from one another. They are at first rounded, almost spherical, with diameters averaging 8.5μ to 9μ, but after the colony of dissociated myxamoebae has been allowed to stand for 15 to 30 minutes the organisms tend to become more elongated, show a differentiation into the endoplasmic and ectoplasmic areas, and begin a slow but perceptible amoeboid movement which marks the beginning of a new organization, or reorganization, of the myxamoebae into new fruiting masses. On the other hand, if a similar pseudoplasmodium is killed and stained in situ and then removed from the agar surface and broken apart, the myxamoebae are seen to be not round but elongated and somewhat angular in outline. As would be expected, the long axes are oriented in the direction of movement. The average dimensions of the myxamoebae at this stage are about 12μ to 13μ by 5μ to 6μ. Movement of the migrating pseudoplasmodium as a unit is obviously accomplished by the concerted and, at the same time, independent action of the countless individual myxamoebae of which it is composed.

In the Acrasieae the production of slime is characteristic, but in *Dictyostelium discoideum* it manifests itself in an earlier stage and in a somewhat different manner. As the migrating pseudoplasmodium moves over the agar surface it produces and leaves behind a trail of slime which persists and can be seen leading away from any migratory mass or mature sorocarp. The material is quite tenacious, for when a sorocarp or pseudoplasmodium is removed, invariably a portion of the slime streak, often 2 to 3 cm in length, remains attached to it.

Plate 2, *D*, shows a pseudoplasmodium with a trail of slime clearly marking the path it has traveled; cutting diagonally across the lower right-hand corner of the same photograph is the trail of another pseudoplasmodium. Embedded in the slime streaks are scattered myxamoebae that have been left behind; microscopically they appear as vacuolated cells of irregular shape. As will be seen later, the number of myxamoebae thus left behind varies with the rate of progress made by the migrating structure.

Regularly the migration pseudoplasmodium produced by an aggregating colony of myxamoebae remains as a unit as it creeps over the plate, and forms a single sorocarp. However, the mass not uncommonly divides, either leaving behind a mass of myxamoebae which produces an independent fructification or splitting into two masses each of which continues to move as a typical migratory structure. Secondary divisions of these pseudoplasmodial masses have occasionally been observed. In order to study their habits to best advantage, the migrating structures were removed from the cultures in which they grew and transplanted to fresh plates where they were less crowded and where their movements could be followed more easily.

In contrast to this splitting of a pseudoplasmodium into more or less equal masses, in older cultures there frequently occurs a gradual fragmentation of the entire structure. In such cases comparatively small masses of myxamoebae are cast aside, and these proceed to develop into small atypical sorocarps at the point where they are cut loose from the larger, parent structure. A large pseudoplasmodium may in this way give rise to a whole series of miniature fruiting bodies lining its path; and intermixed with the sorocarps countless myxamoebae remain stranded, either singly or in groups of varying size. Just what brings this condition about is not entirely clear, but it is no doubt influenced by the drying out of the agar and perhaps through the accumulation of waste products as well.

In cultures grown at 18° to 25° C. the migration pseudoplasmodia are formed and behave in the manner described above. When cultures are grown at 30° to 32° the pseudoplasmodia are formed in the same way but behave very differently. In such cultures the majority never leave the bacterial colony in which they are formed, but proceed to form sorocarps at the points where the aggregates develop. Sorocarps formed under such conditions, however, are not unlike those which develop from migrating pseudoplasmodia. Further studies are in progress concerning the growth and behavior of the species in response to changes in its environment.

SOROCARP FORMATION

Following the period of migration described above, the pseudoplasmodium ceases forward movement and builds an erect fruiting structure which, in accordance with Harper's usage, may be called the sorocarp. This development is shown in a series of photographs (pl. 2, *E–L*), all of which, with the exception of plate 2, *G*, are side views of successive stages.

The first evidence of this development is a relative shortening and thickening of the pseudoplasmodium. Forward movement of the mass becomes progressively slower until it ceases altogether. The myxamoebae, however, continue to crowd together, and the whole

structure takes on a more rounded appearance (pl. 2, *E*). In plate 2, *F*, this tendency has progressed further, and what has been the pointed anterior end now appears as a raised knob at one side; the whole colony is here becoming definitely transformed into an upright structure. Plate 2, *G*, shows a slightly more advanced stage as viewed from above. The structure presents a rounded, almost symmetrical form, with the apex of the developing sorocarp lifted above the mass.

In plate 2, *H*, the structure is more erect and is becoming definitely differentiated into sorophore and spore-forming parts, and it is in structures presenting this appearance that the early stages of sorophore formation are to be found. As development continues and the sorophore becomes longer and sufficiently strong to support the weight, the mass of myxamoebae slowly begins its ascent. In plate 2, *I*, this is clearly shown; the basal portion of the sorophore is complete and is securely anchored to the substratum, and the spore-forming mass is quite removed from it. This particular figure, however, is somewhat atypical in that it shows no pronounced apical region where sorophore formation is taking place. In the developing sorocarp of *Dictyostelium mucoroides*, as pointed out by Harper (*2*) and as seen in culture, the pseudoplasmodium extends down the sorophore as a band either applied to one side or coiled spirally around it, and may form a continuous stream between the substratum and the sorogenic mass. Such a condition has not been observed in *D. discoideum;* the pseudoplasmodium, or spore-forming mass, climbs the developing sorophore as a compact unit.

Plate 2, *J*, is a somewhat later stage and more nearly presents the usual picture. The apical region is composed of undifferentiated myxamoebae, which later become extremely vacuolated and contribute to sorophore development. The myxamoebae making up the bulbous mass below are destined to form spores; in fact, those nearest the periphery have already become differentiated at this stage. The fact that the stalk and base in this figure are heavier in proportion to the size of the sorocarp as a whole than usually is the case is due to exposure to the dry air of the laboratory preparatory to and during the process of photographing. A still more advanced stage is shown in plate 2, *K*, in which the relative proportions of parts in the developing structure are more nearly normal. The well-formed apical papilla indicates that active sorophore formation is in progress. Mature sorocarps are shown in plates 1, *A*, and 2, *L*, all the myxamoebae having become differentiated either into stalk cells or spores.

The mature sorocarp is typically a symmetrical structure, consisting of a disklike cellular base which envelops and supports the base of the stalk, an unbranched sorophore of similar nature which is relatively thick and rigid below but tapers rather evenly to a thin terminal region above, and a rounded apiculate or lemon-shaped sorus. Such an expanded disklike base has not hitherto been described in the Acrasieae. When seen from above, this basal disk is roughly circular in outline, and the upright sorophore arises perpendicularly from the central region of it (pl. 3, *D* and *F*). When viewed from the side, the structure presents a somewhat conical appearance, with a more or less centrally placed cuplike depression into which the base of the sorophore fits (pl. 3, *E*).

What actually happens is that the basal portion of the upright sorophore is first formed and then the basal disk is built outward from it

at the same time that it continues to be built upward. When a young fruiting mass such as that shown in plate 2, *H*, is crushed, it is found that the basal part of the sorophore is in process of formation, and extending outward from it on all sides a band of myxamoebae are cut off by what is apparently a slime sheath not unlike that which extends upward through the mass during sorophore formation. Inside this basal sheath, the cells nearest the upright sorophore are differentiated first and later those further removed are differentiated.

At the periphery of the completed disk the highly vacuolated and horizontally flattened cells form a single layer (pl. 3, *F*). The disk becomes thicker in cross section nearer the sorophore, with the cells piled one above the other. Bordering the sorophore a sleevelike layer of disk cells extends upward for some distance (pl. 3, *E*). The whole structure is enclosed in an envelop of slime which is continuous with that of the sorophore, and extending out from one side of the disk is the trail of slime laid down by the migrating pseudoplasmodium, shown in plate 3, *D*, as a thin line curved about the base.

The sorophore is composed of a slightly bulbous base (pl. 3, *D*), which is enclosed in the basal disk described above, and a cylindrical, elongated tapering shaft which supports the sorus. Its structure is like that of *Dictyostelium mucoroides* (pl. 3, *B*, *C*, *D*, and *F*), although it is more rigid and is almost invariably formed perpendicular to the substratum to which it is attached. Its greater rigidity is due to two factors: (1) In relation to height it has a greater basal diameter and the degree of tapering is greater than in *D. mucoroides*, thus adding to its strength; and (2) the expanded cellular base rests squarely upon the substratum, thus giving the sorophore a much firmer support than in the more common species, where it is anchored by slime only.

As noted above, the bulbous base of the sorophore is formed first, and extending upward from it in the central region of the young sorocarp is a tubular slime sheath which is formed in advance of the vacuolated cells and in which these cells become packed as growth of the sorophore progresses. This sheath is continuous with the sorophore below and the greater part of it is of the same diameter; the terminal region, however, is regularly expanded into a funnellike structure. A similar structure is present in other *Dictyostelia*, and figures of it in *Dictyostelium mucoroides* have been given by Brefeld (*1, pl. III, fig. 21*) and Harper (*2, pl. 6, fig. 14*). Brefeld's figure shows the slime sheath formed for some distance beyond the enclosed, vacuolated cells; and, except in the absence of an expanded terminus, it agrees with structures commonly seen in young, rapidly developing sorocarps of *D. discoideum*. As to the origin of this sheath, it is not clear whether it is secreted by cells that are destined to become stalk

EXPLANATORY LEGEND FOR PLATE 3

A.—Terminal region of developing sorocarp, showing the funnel-shaped sheath formed in advance of sorophore formation. The apical papilla and the region surrounding the top of the sorophore are composed of amoeboid cells. Below, spores are already differentiated. × 320.

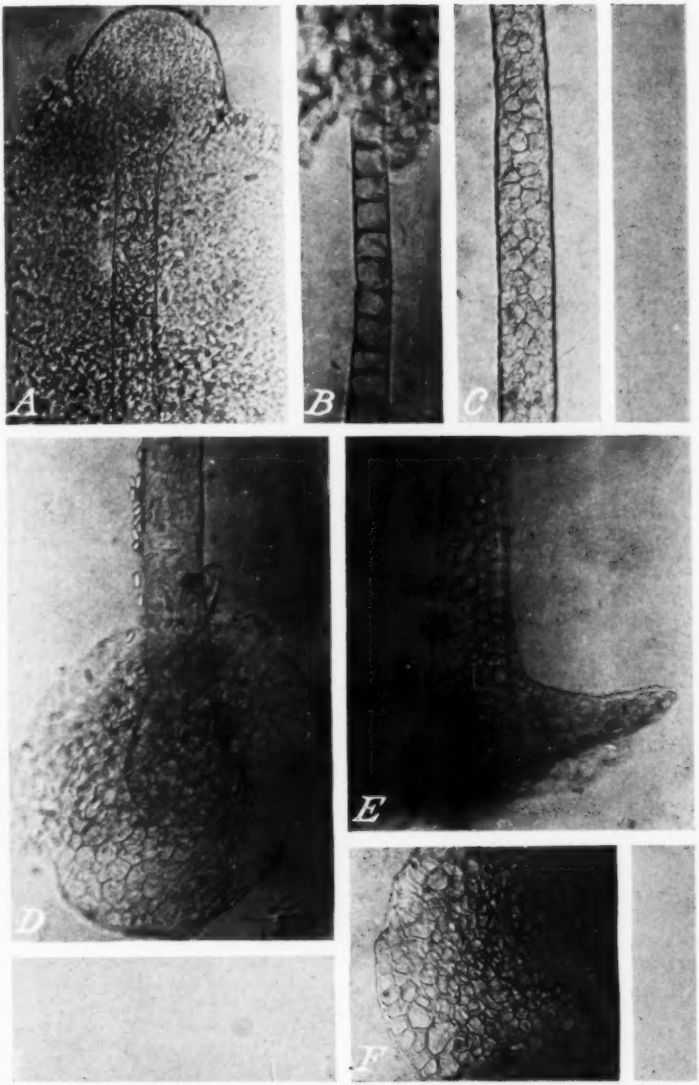
B.—Terminal region of a sorophore, consisting of cells in a single row or superimposed on other cells. × 900.

C.—Median region of a sorophore. × 320.

D.—Basal part of a sorocarp, showing the swollen base of the upright sorophore and the expanded basal disk from which it arises. The slime streak remained attached and is shown as a line curved about the base of the sorophore. × 320.

E.—Basal part of a sorocarp, showing the conical shape of the basal disk and how the end of the sorophore fits into and is attached to it. × 320.

F.—Basal disk as viewed from above, showing the end of the sorophore as a circular structure in the upper right corner of the figure. × 320.



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cells or by the surrounding cells, which later form spores. In either case it serves to separate the two groups of cells before there is any evident differentiation of either.

In plate 3, *A*, the terminal region of a more advanced sorocarp than those just under consideration is shown, and in it, in contrast to the condition in younger, rapidly developing structures, the expanded portion is just above the rounded, vacuolated cells, which later become compacted together to form a rigid sorophore. Above the developing sorophore is the apical papilla of undifferentiated amoeboid cells which contribute to its further extension. Just below and surrounding the terminal region of the sorophore are other undifferentiated myxamoebae; whether they all ultimately become spores or whether some of them join the apical mass and become stalk cells has not been determined.

In the lower part of the picture the outlines of spores already formed can be seen. Once the mass of myxamoebae has become separated from the substratum (*pl. 2, I, J*) and is ascending the sorophore as it develops, the myxamoebae at the periphery of the mass in a centrally located band begin to be differentiated into spores, and as this sorogenic mass rises continually higher on the developing sorophore more and more spores are formed. If a sorocarp such as that shown in pl. 2, *K*, is studied in a drop of water, a band of spores is seen completely encircling the developing head; if as many spores as possible are washed away and the structure is crushed under a cover slip, it becomes evident that beneath the layer of spores is a mass of cells in stages of differentiation into spores and that around the sorophore is a cylinder of cells still amoeboid which carries the mass upward. Gradually the cells in the apical region are used up in sorophore formation, and those surrounding it become spores to complete the fruiting structure.

TABLE 1.—*Comparative dimensions of parts of sorocarps of Dictyostelium discoideum from 3-week-old dung-agar cultures*

Size of sorophore						
Diameter of expanded base	Length	Portion above basal swelling		Terminal region		Diameter of sorus
		Diameter	Cells in cross diameter	Diameter	Cells in cross diameter	
μ	μ	μ	*Number*	μ	*Number*	μ
200	1,800	42	5–6	8.5	1, 1, and 2 [1]	225
200	2,350	38	5	9	do [1]	200
210	2,210	39	5	7.5	1	230
240	2,510	45	6	9.2	1 and 2	275
205	2,250	41	5	7.5	1	240
185	2,050	32	4	7	1	225
210	2,420	37	5	8.5	1, 1, and 2 [1]	300
325	2,850	70	9–10	14	2	350
270	2,500	48	7–8	10.2	2	250
225	2,440	42	6	8.5	1 and 2	275
200	2,240	38	5–6	9	do	225
300	2,625	60	8	10	2	325

[1] The sorophore was usually 1 cell but occasionally 2 cells wide.

When sorocarps in large numbers are observed, one is impressed with the relation in size that exists between their different parts, for example, the diameter of the basal disk, sorophore, and sorus. This relationship is not exact, to be sure, but within limits it does exist. In table 1 are given the measurements of a group of sorocarps picked from dung-agar cultures 3 weeks old. Dimensions of the sori were taken in situ, and the sorocarps were then removed to slides, where other measurements were made. Roughly the ratio of the diameter of the expanded disklike base to the diameter of the sorophore just above the swollen base is 5 to 1 and the ratio of the diameter of the sorophore at this point to its diameter in the terminal region is likewise approximately 5 to 1. The relationship between length and diameter is not so regular, but in general the longer sorophores have the greater diameters, arise from the larger bases, and bear the larger sori.

In *Dictyostelium discoideum*, as in other Acrasieae, there is a striking relation between the diameter of the sorophore in the terminal region and the size of the sorus. And in fructifications where the sorophore terminates in a single row of cells, the shape of these cells varies with the volume of the spore mass. Harper (*2*) has made a detailed study of this matter in *D. mucoroides*, and the writer's observations on *D. discoideum* agree closely with his. If the sorus is very small the cells are narrow and elongated, 15μ to 18μ by 3μ to 3.5μ, or with the long and short diameters in the ratio of 5 to 1; if larger, the cells are more nearly isodiametric; and if still larger, the cells are horizontally flattened, 3μ to 4μ by 9μ to 10μ, or with the long and short diameters in the ratio of 1 to 3. In cases where the sori are especially large, the sorophores are 2 or rarely 3 cells wide and the cells are of approximately equal width and length. Thus it would seem that the size of the sorophore is the essential thing; it must be of a size and strength sufficient to support the spore mass, and the cells that go into the making of it become modified in such a way as to attain this objective.

Sorocarps regularly develop at right angles to the substratum or other support to which they are anchored. In the usual culture they are produced upon the agar surface and are built up vertically. If grown in inverted plates they are built vertically downward. If cultures are grown in uneven light and as a result migrating pseudoplasmodia climb up the side of the culture dish, the resulting sorocarps develop outward horizontally. In cultures grown in uneven light or temperature there is no pronounced inclination of the sorophores toward either light or warmer temperature.

True branching has not been observed in *Dictyostelium discoideum*. On several occasions structures have been seen which, under low magnification, appeared to be branched sorophores. But in each case, on closer examination, the "branched" structure was seen to be composed of two sorocarps complete in every detail, one of which was anchored to the substratum while the other was anchored to the upright sorophore of the first. The "branch" possessed a typical expanded base, which was somewhat curved around the supporting sorophore and was securely anchored to it by an envelop of slime. In regard to such compound structures the question arises as to whether the supporting and the supported sorocarps arise from the same colony of aggregating myxamoebae. The evidence indicates that this is not the case but that the branch originated in a later pseudoplasmodium which climbed up an established sorophore, just

as it would have climbed up the side of the culture dish, and then proceeded to form a sorocarp.

SUMMARY

A new species of *Dictyostelium* is described, for which the name *Dictyostelium discoideum* is proposed.

Following the aggregation of the myxamoebae, the pseudoplasmodium becomes a compact cylindrical mass and moves for a greater or less distance over the culture plate before developing a sorocarp. This migratory stage, which has not been previously reported, is termed the migration pseudoplasmodium.

The mature sorocarp differs from that of the more common species in possessing a cellular basal disk, which surrounds and supports the base of the sorophore. In addition, the sorophore is more rigid and tapers more evenly than in other species.

LITERATURE CITED

(1) Brefeld, O.
1869. dictyostelium mucoroides. ein neuer organismus aus der verwandschaft der myxomyceten. Abhandl. Senckenb. Naturf. Gesell. 7: 85–107, illus.

(2) Harper, R. A.
1926. morphogenesis in dictyostelium. Bull. Torrey Bot. Club 53: 229–268, illus.

(3) Krzemieniewski, H. S., and Krzemienski, S.
1927. z. mikroflory gleby w polsce. (contribution à la microflore du sol en pologne.) Acta Soc. Bot. Poloniae 4: 141–144.

(4) Olive, E. W.
1902. monograph of the acrasieae. Boston Soc. Nat. Hist. Proc. 30: 451–514, illus.

(5) Pinoy, E.
1907. rôle des bactéries dans le dévelopement de certains myxomycètes. Ann. Inst. Pasteur 21: [622]–656, 686–700, illus.

(6) Raper, K. B., and Thom, C.
1932. the distribution of dictyostelium and other slime molds in soil. Jour. Wash. Acad. Sci. 22: 93–96.

(7) Skupienski, F. X.
1920. recherches sur le cycle évolutif des certains myxomycètes. 81 pp., illus. Paris.

(8) Thom, C., and Raper, K. B.
1930. myxamoebae in soil and decomposing crop residues. Jour. Wash. Acad. Sci. 20: 362–370.

(9) Tieghem, P. van.
1880. sur quelques myxomycètes a plasmode agrégé. Bull. Soc. Bot. France 27: 317–322.

EFFECT OF TEMPERATURE OF ARTIFICIAL DRYING ON DIGESTIBILITY AND AVAILABILITY OF NUTRIENTS IN PASTURE HERBAGE [1]

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INTRODUCTION

Mechanical driers are being used in the artificial preservation of forage crops in certain sections of the United States. The future of this method of preserving crops for winter feeding depends largely on the development of machines that will dry forage efficiently and economically without affecting its nutritive value.

REVIEW OF PREVIOUS INVESTIGATIONS

As a result of research with a rotary triple-drum drier (Ardrier) Clyde (*4, 5*) [2] has been able to increase the efficiency and drying capacity of the drier by reducing the air flow and increasing the initial temperature of the heating gas. By increasing the initial temperature from an average of 932° to 1,451° F., it was possible to increase the rapidity with which the moisture was evaporated from the hay and to reduce the exhaust temperature from 291.7° to 237°. Barr (*2, 3*) working with the Louisiana station rotary single-drum drier reported that the lowest efficiencies (when drying green forage) were obtained when the initial gas temperature ranged from 1,100° to 1,200° F. and that the highest efficiencies were obtained at 1,850°. He found that with the highest initial heat the exhaust temperature maintained itself at 240° and the forage had a temperature of 198°. The extent to which the temperature of drying may be raised to effect a greater and more rapid evaporation of moisture without rendering valuable feed nutrients unavailable is not apparent. The experiment described herein was undertaken to determine the effect of increasing the temperature of drying on the nutritive value of the herbage.

Hart, Kline, and Humphrey (*11*) found that the nutrients of green alfalfa exposed to temperatures of 480° to 535° C. in a Koon-type drier for 40 seconds were no less digestible than those of similar alfalfa dried in the field. Woodman and his associates (*20*) concluded that the high nutritive value of young pasturage is not appreciably depressed by artificial drying with steam at 100° C. Pasture grass dried by direct heat in a kiln chamber at 115° for 3 hours was only slightly less digestible than similar herbage dried at the temperature of steam. They report that the grass, while in the chamber, at no time reached a temperature of over 90°.

[1] Received for publication Nov. 19, 1934; issued April 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 163.

Watson and Ferguson (*18*) compared the digestibility of pasturage that had been dried at different temperatures in a band drier and in a pneumatic-conveyor drier with fresh green herbage. In the band drier, at an inlet temperature of 200° C., the herbage was dried to 10-percent moisture in 20 minutes with no effect on the digestibility of the various constituents, except on that of the crude protein, which was slightly depressed. With similar herbage, dried in the conveyor drier at a temperature of 600° for 15 seconds, a depression of the digestibility of the constituents of the grass was noted, particularly the digestibility of crude protein which was decreased markedly. Watson and Ferguson (*19*) reported that hay of excellent quality and high digestibility was made by artificial drying in a stack through which hot air was blown. The temperature of the hay was maintained at an average of 100° to 110° F. for 45 hours during the drying process. Honcamp (*15*) reported that artificial drying of grass at low temperatures caused no loss in the raw or digestible nutrients. When artificial drying was accomplished by hot fire gases a considerable reduction of protein digestibility occurred. He did not, however, specify what temperatures were used.

Several investigators (*14, 16, 18, 20*) have reported the digestibility coefficients of the nutrients in artificially dried young grass. In most instances the materials studied have been dried under different conditions. To facilitate comparison these coefficients are assembled in table 1.

TABLE 1.—*Coefficients of digestibility of artificially dehydrated pasture herbage, as reported by several investigators*

Treatment of grass	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Ash	Investigators
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
2-week-old grass dried in oven at 150° F. for 10 hours.	67.57	74.92	74.55	21.90	72.68	32.48	Hodgson and Knott (*14*).
7- to 10-day-old grass artificially dehydrated in Ardrier.	72.4	71.0	81.6	46.4	73.0	38.5	Newlander and Jones (*16*).
Young grass dried at 600° C. for 40 seconds.	65.90	58.94	75.96	55.76	68.09	.....	Watson and Ferguson (*18*).
Young grass dried at 100° C. for 3 hours.	.....	78.2	80.4	73.7	81.0	.....	Woodman, Bee, and Griffith (*20*).

EXPERIMENTAL PROCEDURE

The pasture herbage used in this investigation was a mixture of English ryegrass (*Lolium perenne*), Italian ryegrass (*L. multiflorum*), and white clover (*Trifolium repens*). The pasture was seeded in the early spring of 1933 on fertile bottom-land soil on the Western Washington Experiment Station at Puyallup. On April 17, after the seeding had made a good start, 300 pounds of sodium nitrate and 450 pounds of superphosphate per acre were applied. Due to the abundant and well-distributed rainfall a luxuriant and uniform growth was maintained throughout May, June, and July, when the samples were taken. The herbage was rather free from weeds and contained approximately 20 percent of white clover and 40 percent of each of the ryegrasses.

The pasture was divided into three plots, one of which was cut each week. The grass represented 3 weeks' growth when cut. Grass that was to be fed green was cut each day, run through a chopper, and fed twice daily. Grass that was to be dried, either artificially or in the sun, was cut as soon as the dew was off in the morning, collected immediately, run through a silage cutter, and spread out to wilt overnight, and dried the following day. Curing in the sun was accomplished by spreading the grass thinly on a canvas and exposing it to the sun and indirect sunlight for 18 hours on the average. The grass was turned occasionally to insure uniform exposure, but was not exposed to rain or dew and suffered little or no loss of leaves. Artificial drying was accomplished with an experimental direct-heat rotary single-drum drier.[3] Diesel oil was used as a source of fuel. The temperature of the gas at the outlet end of the drier was used as an index of the drying temperature, grass being dried at four different temperatures. The inlet temperature and the rate of speed at which the grass passed through the machine remained about the same for the four different rations dried, the only varying factor being the amount of material in the machine. The amount of material in the machine at any one time regulated the exhaust-gas temperature. The grass was dried at outlet temperatures of 250°, 300°, 350,° and 400° F., with an average variation of not over 5° above or below these temperatures. The wilted herbage, having an average dry-matter content of 19.5 percent, passed through the drying machine in from 2 to 5 minutes.

Six digestion and mineral-balance trials were conducted with a group of three sheep, each of the six rations of pasture grass described above constituting a trial. The sheep were range yearling wethers of unknown history. They were conditioned to the metabolism crates and rations before the experimental trials were begun. During each trial the three sheep were confined to individual metabolism crates (fig. 1). The crates were patterned after those described by Forbes (*6*) but with certain modifications to meet specific needs. Each crate had a floor area of 20 square feet. A manger and yoke were built in one end of the crate so that the sheep could be stanchioned while eating (figs. 2 and 3). Water and salt were accessible through openings in the rear end of the crate (fig. 2). The crate had two false bottoms, one of ⅞-inch mesh galvanized wire on which the sheep stood and which permitted the excreta to pass through, and the other of ¼-inch mesh galvanized wire which caught the feces and allowed the urine to pass through. The urine was caught on a funnel-shaped pan and conveyed to glass receptacles (fig. 4).

The procedure outlined by Forbes and Grindley (*7*) for conducting digestion trials was followed in this experiment. The digestion trial for each type of feed lasted 3 weeks. The preliminary period was 7 days, while the collection period continued for 14 days. The sheep were weighed for 3 successive days at the beginning and end of each trial.

The freshly cut herbage was thoroughly mixed, and weighed amounts were fed to the sheep. At the same time duplicate samples were taken for dry matter and chemical determinations. In the trial

[3] The writers wish to express appreciation for cooperation given by M. S. Grunder, agronomist, Western Washington Experiment Station, who built the artificial drying machine and supervised its operation in the preparation of the dried grass used in the experiment.

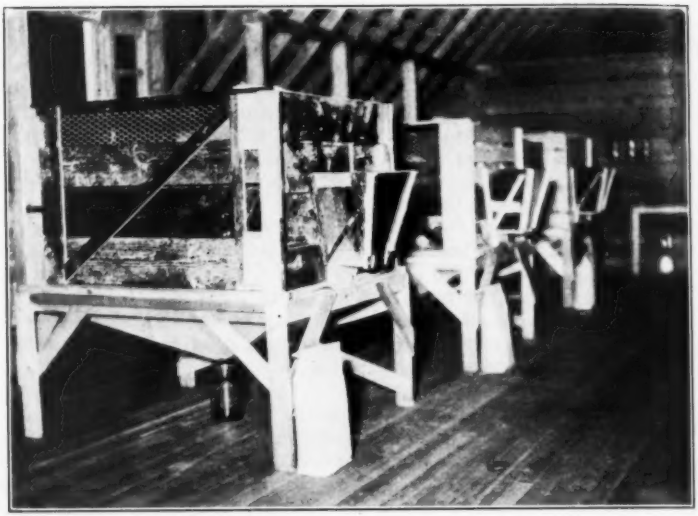

FIGURE 1.—Individual metabolism crates used for sheep.

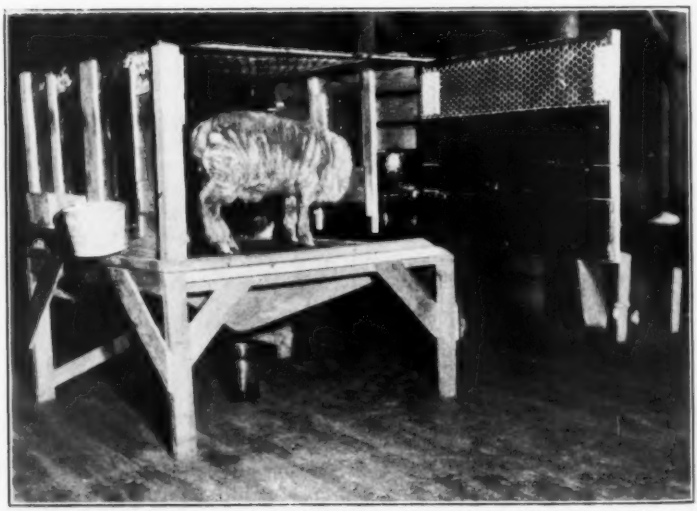

FIGURE 2.—Metabolism crate open and showing salt and water receptacles, and wether stanchioned as for feeding.

with the freshly cut herbage the refused feed was weighed daily and sampled for dry matter and chemical analysis. There was no refused feed during the trials in which the sun-cured grass or the artificially dried grass rations were fed.

At the beginning of the trials in which the sun-cured grass ration and the four artificially dried grass rations were fed, a sufficient amount of the material from each ration to last the entire trial was weighed out, thoroughly mixed, and divided into three piles. Weighed individual feedings, which were the same throughout the trial, were made up from the three piles and stored in paper bags. At regular intervals samples from each of the three piles were saved for chemical analysis and for color determinations.

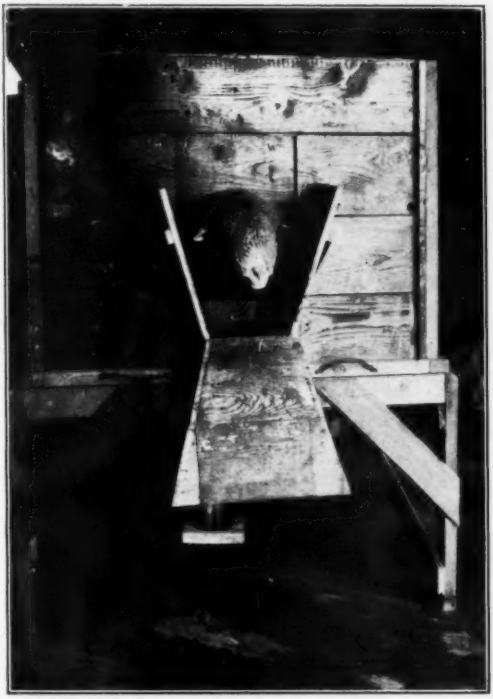

FIGURE 3.—Metabolism crate with wether stanchioned and the front of the manger down to facilitate cleaning.

The sheep were fed twice daily, at which time they were fastened in the yokes to avoid losing feed in the crates. Fresh spring water containing negligible amounts of calcium and phosphorus was provided at all times and salt was fed daily.

At the end of the experimental day the feces were collected, thoroughly mixed, weighed, and 50 percent of the daily excretion was saved for chemical analysis. The samples were collected in a thymol-coated receptacle and stored in a refrigerator at 22° F. The daily excretion of urine was mixed, weighed, and a 20-percent aliquot sample saved for analysis. The samples were stored in thymol-coated air-tight glass bottles and preserved by adding a thymol chloroform mixture at the rate of 2 cc per liter. A 10-percent acetic acid solution was added at the rate of 1 cc per liter to prevent precipitation. At the close of the collection period the daily aliquots of urine and feces of each sheep were mixed and sampled for chemical analysis. The analyses of the feeds, feces, and urine were made by the usual methods as outlined by the Association of Official Agricultural Chemists (*1*). The color determinations

of the samples of the dried herbage were made according to the method described by Nickerson (*17*).[4]

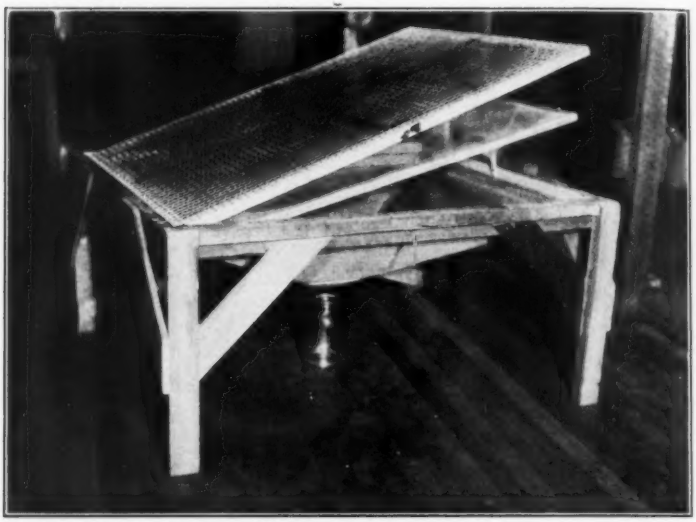

FIGURE 4.—Platform of metabolism crate showing the upper screen on which the animal stands, the lower screen which retains the feces and allows the urine to pass through, and the sloping metal bottom which drains the urine into the glass receptacle.

EXPERIMENTAL RESULTS

COMPOSITION OF FEED

The chemical composition of the six rations of pasture grass used in this experiment is shown in table 2.

TABLE 2.—*Chemical composition of rations of pasture grass used in the experiment (dry-matter basis)*

Rations	Dry matter	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash	Nitrogen	Calcium	Phosphorus	Ca/P
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Ratio*
Green grass, no treatment	23.96	16.36	21.46	3.92	45.99	12.27	2.62	0.761	0.484	1.57
Sun-cured grass, exposed 18 hours	92.16	18.82	20.54	3.46	44.62	12.56	3.01	.732	.647	1.13
Grass artificially dried at exhaust temperature of—										
250° F.	92.41	17.50	20.21	4.59	46.52	11.18	2.80	.729	.516	1.41
300° F.	92.72	17.78	20.98	5.09	43.79	12.36	2.84	.762	.555	1.37
350° F.	92.56	18.19	20.98	4.26	44.90	11.67	2.91	.670	.517	1.30
400° F.	93.18	17.98	22.88	5.04	42.46	11.64	2.88	.689	.583	1.18

The various dried-grass rations are very uniform in composition, indicating that each sample was truly representative of the herbage. The slight increase in the crude-fiber content of the grass dried at the

[4] Color determinations were made by the Bureau of Agricultural Economics, U. S. Department of Agriculture.

exhaust-gas temperature of 400° F. may indicate slight burning of the more leafy material as it passed through the machine. Upon looking into the exhaust end of the drier it was apparent that some burning was taking place at this high drying-temperature. The calcium and phosphorus content of this herbage is high but is in close agreement with that of other grass produced on adjacent fields at this station. (*10, 13, 14.*)

COMPOSITION OF EXCRETA

In table 3 is given the composition of the urine and feces of the animals used in the six metabolism trials. There is a high degree of uniformity in the composition of the organic and inorganic constituents of the excreta of the individuals during each of the test periods. The dry-matter content of the feces of sheep no. 1 was considerably lower than that of the others throughout each experiment. This condition did not, however, affect the composition of the dry matter of the feces or the ability of the animal to digest nutrients. The grass dried at 350° and 400° F. had a more laxative effect upon the animals than the other rations.

TABLE 3.—*Chemical composition of feces and urine of sheep receiving rations of green, sun-cured, and artificially dehydrated pasture grass dried at different temperatures*

Sheep no., and material analyzed	Dry matter	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash	Nitrogen	Calcium	Phosphorus
GREEN GRASS, NO TREATMENT									
Sheep 1:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Feces	42.09	15.21	17.45	3.82	37.12	26.40	2.43	2.395	1.4111
Urine							1.01	.0117	.00122
Sheep 2:									
Feces	53.57	15.12	18.98	4.44	36.48	24.98	2.43	2.388	1.371
Urine							.839	.00418	.000936
Sheep 3:									
Feces	54.76	15.30	18.23	3.47	37.44	25.56	2.45	2.410	1.434
Urine							.914	.0091	.00079
SUN-CURED GRASS, EXPOSED 18 HOURS									
Sheep 1:									
Feces	46.86	17.02	13.27	5.21	32.44	32.06	2.72	2.675	2.010
Urine							1.486	.00404	.000855
Sheep 2:									
Feces	53.80	16.53	14.16	4.45	32.59	32.27	2.64	2.698	1.971
Urine							1.256	.00113	.000927
GRASS ARTIFICIALLY DRIED AT 250° F.									
Sheep 1:									
Feces	34.74	18.36	15.03	6.56	32.66	27.39	2.94	2.656	1.856
Urine							1.759	.00374	.00107
Sheep 2:									
Feces	55.37	17.30	15.86	4.02	34.63	28.19	2.77	2.788	1.913
Urine							1.332	.00176	.000911
Sheep 4:									
Feces	52.06	18.12	14.69	5.88	32.78	28.53	2.90	2.699	1.925
Urine							1.562	.00310	.00110

TABLE 3.—*Chemical composition of feces and urine of sheep receiving rations of green, sun-cured, and artificially dehydrated pasture grass dried at different temperatures*—Continued

GRASS ARTIFICIALLY DRIED AT 300° F.

Sheep no., and material analyzed	Dry matter	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash	Nitrogen	Calcium	Phosphorus
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Sheep 1:									
Feces	34.00	18.65	15.44	5.65	33.25	27.01	2.98	2.704	1.883
Urine							1.813	.00247	.00103
Sheep 2:									
Feces	46.94	18.12	15.81	5.05	33.34	27.68	2.90	2.750	1.835
Urine							1.477	.00199	.000835
Sheep 4:									
Feces	50.39	18.50	16.79	3.80	32.16	28.75	2.96	2.918	1.918
Urine							1.846	.00215	.00116

GRASS ARTIFICIALLY DRIED AT 350° F.

Sheep 1:									
Feces	27.36	20.66	14.91	6.07	32.01	26.35	3.31	2.537	1.749
Urine							1.837	.00224	.000935
Sheep 2:									
Feces	41.64	19.87	14.96	4.03	34.46	26.68	3.18	2.575	1.768
Urine							1.531	.03344	.000756
Sheep 4:									
Feces	36.20	20.38	14.75	3.70	34.06	27.11	3.26	2.618	1.805
Urine							1.779	.00296	.000940

GRASS ARTIFICIALLY DRIED AT 400° F.

Sheep 1:									
Feces	30.05	24.93	14.13	4.00	32.62	24.32	3.99	2.193	1.572
Urine							1.499	.00176	.00127
Sheep 2:									
Feces	39.77	24.06	15.00	2.75	34.21	23.98	3.85	2.204	1.541
Urine							1.315	.00134	.00101
Sheep 4:									
Feces	37.85	24.41	14.57	2.48	33.86	24.68	3.91	2.304	1.662
Urine							1.384	.00158	.00101

There was a higher percentage of protein in the feces of the sheep when receiving grass dried at 400° F., indicating that they were not digesting this constituent so completely. There was a rather high percentage of nitrogen in the urine but the excretion of calcium and phosphorus from this source was very small.

APPARENT DIGESTIBILITY OF NUTRIENTS

The rations fed to the sheep were sufficient to meet their requirements for digestible nutrients as recommended in the Henry and Morrison standards (*12*). Due to the high percentage of protein in the grass the intake of that constituent was more than sufficient to meet the daily requirements of the wethers. As shown in table 4 the sheep made slight gains in live weight during each digestion trial except sheep no. 3 when receiving green grass. This animal did not adjust itself to the conditions of the experiment and therefore did not eat well. It was necessary to substitute another sheep for it in the later experiments.

TABLE 4.—*Average weight of each sheep during the first 3 days and the last 3 days of the 21-day period on each of the rations*

Ration	Average weight of—								
	Sheep no. 1—			Sheep no. 2—			Sheep no. 3—		
	Initial	Final	Gain	Initial	Final	Gain	Initial	Final	Gain
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Green grass, no treatment	101.8	105.8	4.0	102.8	107.5	4.7	97.8	95.2	[1] −2.6
Sun-cured grass, exposed 18 hours	105.2	107.2	2.0	108.5	111.2	2.7	.....	.....	.....
Grass artificially dried at exhaust temperature of—									
250° F	109.2	112.0	2.8	114.7	115.5	.8	[2] 113.7	[2] 116.2	[2] 2.5
300° F	113.0	113.8	.8	117.7	120.0	2.3	[2] 115.5	[2] 119.5	[2] 4.0
350° F	114.2	114.8	.6	122.3	126.0	3.7	[2] 122.2	[2] 124.8	[2] 2.6
400° F	115.3	117.3	2.0	125.3	129.2	3.9	[2] 125.0	[2] 127.8	[2] 2.8

[1] Loss.
[2] Sheep no. 4 substituted for sheep no. 3, which went off feed in second trial.

TABLE 5.—*Dry matter and nutrients ingested, voided, and digested during a 14-day period by sheep receiving rations of green, sun-cured, and artificially dehydrated grass*

GREEN GRASS, NO TREATMENT

Sheep no., and items compared		Dry matter	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash
Sheep 1:							
Fed	grams	19,540.0	3,196.7	4,193.3	766.0	8,986.4	2,397.6
Refused	do	817.2	148.7	184.7	27.5	350.3	105.9
Consumed	do	18,722.8	3,048.0	4,008.6	738.5	8,636.1	2,291.7
Voided	do	5,143.4	782.3	897.5	196.5	1,909.2	1,357.9
Digested	do	13,579.4	2,265.7	3,111.1	542.0	6,726.9	933.8
Digested	percent	72.5	74.3	77.6	73.4	77.9	40.7
Sheep 2:							
Consumed	grams	20,130.0	3,293.3	4,319.9	789.1	9,257.2	70.0
Voided	do	6,517.3	985.4	1,237.0	289.4	2,377.5	1,628.0
Digested	do	13,612.7	2,307.9	3,082.9	499.7	6,880.3	842.0
Digested	percent	67.6	70.1	71.4	63.3	74.3	34.1
Sheep 3:							
Fed	grams	19,160.0	3,134.6	4,111.7	751.1	8,811.7	2,350.9
Refused	do	2,897.0	509.0	677.6	81.4	1,241.6	387.3
Consumed	do	16,263.0	2,625.6	3,434.1	669.7	7,570.0	1,963.6
Voided	do	4,679.8	716.0	853.1	162.4	1,752.1	1,196.2
Digested	do	11,583.2	1,909.6	2,581.0	507.3	5,817.9	767.4
Digested	percent	71.2	72.7	75.2	75.8	76.8	39.1

SUN-CURED GRASS, EXPOSED 18 HOURS

Sheep 1:							
Consumed	grams	15,482.9	2,913.9	3,180.2	535.7	6,908.5	1,944.6
Voided	do	3,710.4	631.5	492.4	193.3	1,203.6	1,189.5
Digested	do	11,772.5	2,282.4	2,687.8	342.4	5,704.9	755.1
Digested	percent	76.0	78.3	84.5	63.9	82.6	38.8
Sheep 2:							
Consumed	grams	15,482.9	2,913.9	3,180.2	535.7	6,908.5	1,944.6
Voided	do	3,791.3	626.7	536.8	168.7	1,235.6	1,223.4
Digested	do	11,691.6	2,287.2	2,643.4	367.0	5,672.9	721.2
Digested	percent	75.5	78.5	83.1	68.5	82.1	37.1

TABLE 5.—*Dry matter and nutrients ingested, voided, and digested during a 14-day period, by sheep receiving rations of green, sun-cured, and artificially dehydrated grass*—Continued

GRASS ARTIFICIALLY DRIED AT 250° F.

Sheep no., and items compared	Dry matter	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash
Sheep 1:						
Consumed ..... grams	15, 524. 9	2, 716. 8	3, 137. 6	712. 6	7, 222. 2	1, 735. 7
Voided ..... do	3, 643. 5	669. 0	547. 6	239. 0	1, 190. 0	998. 0
Digested ..... do	11, 881. 4	2, 047. 8	2, 590. 0	473. 6	6, 032. 2	737. 7
Digested ..... percent	76. 5	75. 4	82. 6	66. 5	83. 5	42. 5
Sheep 2:						
Consumed ..... grams	15, 524. 9	2, 716. 8	3, 137. 6	712. 6	7, 222. 2	1, 735. 7
Voided ..... do	3, 535. 9	611. 7	560. 8	142. 1	1, 224. 5	996. 8
Digested ..... do	11, 989. 0	2, 105. 1	2, 576. 8	570. 4	5, 997. 7	738. 9
Digested ..... percent	77. 2	77. 5	82. 1	80. 1	83. 0	42. 6
Sheep 4:						
Consumed ..... grams	15, 524. 9	2, 716. 8	3, 137. 6	712. 6	7, 222. 2	1, 735. 7
Voided ..... do	3, 708. 2	671. 9	544. 7	218. 0	1, 215. 6	1, 058. 0
Digested ..... do	11, 816. 7	2, 044. 9	2, 592. 9	494. 6	6, 006. 6	677. 7
Digested ..... percent	76. 1	75. 3	82. 6	69. 4	83. 2	39. 0

GRASS ARTIFICIALLY DRIED AT 300° F.

Sheep 1:						
Consumed ..... grams	15, 577. 0	2, 769. 6	3, 268. 0	792. 9	6, 821. 2	1, 925. 3
Voided ..... do	3, 649. 2	680. 6	563. 4	206. 2	1, 213. 4	985. 6
Digested ..... do	11, 927. 8	2, 089. 0	2, 704. 6	586. 7	5, 607. 8	939. 7
Digested ..... percent	76. 6	75. 4	82. 8	74. 0	82. 2	48. 8
Sheep 2:						
Consumed ..... grams	15, 577. 0	2, 769. 6	3, 268. 0	792. 9	6, 821. 2	1, 925. 3
Voided ..... do	3, 632. 7	658. 2	574. 3	183. 4	1, 211. 1	1, 005. 5
Digested ..... do	11, 944. 3	2, 111. 4	2, 693. 7	609. 5	5, 610. 1	919. 8
Digested ..... percent	76. 7	76. 2	82. 4	76. 9	82. 2	47. 8
Sheep 4:						
Consumed ..... grams	15, 577. 0	2, 769. 6	3, 268. 0	792. 9	6, 821. 2	1, 925. 3
Voided ..... do	3, 771. 7	697. 8	633. 3	143. 3	1, 257. 5	1, 084. 4
Digested ..... do	11, 805. 3	2, 071. 8	2, 634. 7	649. 6	5, 563. 7	840. 9
Digested ..... percent	75. 8	74. 8	80. 6	81. 9	81. 6	43. 7

GRASS ARTIFICIALLY DRIED AT 350° F.

Sheep 1:						
Consumed ..... grams	14, 254. 2	2, 592. 8	2, 990. 5	607. 2	6, 400. 2	1, 663. 5
Voided ..... do	2, 948. 6	609. 2	439. 6	179. 0	943. 8	777. 0
Digested ..... do	11, 305. 6	1, 983. 6	2, 550. 9	428. 2	5, 456. 5	886. 5
Digested ..... percent	79. 3	76. 5	85. 3	70. 5	85. 2	53. 3
Sheep 2:						
Consumed ..... grams	15, 550. 1	2, 828. 6	3, 262. 4	662. 4	6, 982. 0	1, 814. 7
Voided ..... do	3, 497. 0	694. 9	523. 2	140. 9	1, 205. 1	933. 0
Digested ..... do	12, 053. 1	2, 133. 7	2, 739. 2	521. 5	5, 776. 9	881. 7
Digested ..... percent	77. 5	75. 4	84. 0	78. 7	82. 7	48. 6
Sheep 4:						
Consumed ..... grams	15, 550. 1	2, 828. 6	3, 262. 4	662. 4	6, 982. 0	1, 814. 7
Voided ..... do	3, 495. 5	712. 4	515. 6	129. 3	1, 190. 6	947. 6
Digested ..... do	12, 054. 6	2, 116. 2	2, 746. 8	533. 1	5, 791. 4	867. 1
Digested ..... percent	77. 5	74. 8	84. 2	80. 5	83. 0	47. 8

GRASS ARTIFICIALLY DRIED AT 400° F.

Sheep 1:						
Consumed ..... grams	14, 023. 6	2, 521. 4	3, 208. 6	706. 8	5, 954. 4	1, 632. 3
Voided ..... do	4, 241. 9	1, 057. 5	599. 4	169. 7	1, 383. 7	1, 031. 6
Digested ..... do	9, 781. 7	1, 463. 9	2, 609. 2	537. 1	4, 570. 7	600. 7
Digested ..... percent	69. 8	58. 1	81. 3	76. 0	76. 8	36. 8
Sheep 2:						
Consumed ..... grams	15, 328. 1	2, 756. 0	3, 507. 1	772. 5	6, 508. 3	1, 784. 2
Voided ..... do	4, 627. 6	1, 113. 4	694. 2	127. 3	1, 583. 1	1, 109. 7
Digested ..... do	10, 700. 5	1, 642. 6	2, 812. 9	645. 2	4, 925. 2	674. 5
Digested ..... percent	69. 8	59. 6	80. 2	83. 5	75. 7	37. 8
Sheep 4:						
Consumed ..... grams	15, 328. 1	2, 756. 0	3, 507. 1	772. 5	6, 508. 3	1, 784. 2
Voided ..... do	4, 545. 4	1, 109. 5	662. 3	112. 7	1, 539. 1	1, 121. 8
Digested ..... do	10, 782. 7	1, 646. 5	2, 844. 8	659. 8	4, 969. 2	662. 4
Digested ..... percent	70. 3	59. 7	81. 1	85. 4	76. 4	37. 1

Table 5 contains data on the dry matter and nutrients ingested, voided, and digested by sheep receiving the different rations. The digestibility coefficients of the individual wethers in each trial are in close agreement for all constituents except the ether extract, which varies considerably.

At the beginning of the experiment with the sun-cured grass, sheep no. 3 went off feed for several days and it was necessary to reject results from it in summarizing the results of the trial.

Table 6 shows the apparent digestibility of the different rations.

TABLE 6.—*Apparent digestibility of green, sun-cured, and artificially dehydrated grass*

Ration	Sheep	Dry matter	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Green grass, no treatment	3	70.4	72.3	74.6	70.5	76.3	37.8
Sun-cured grass, exposed 18 hours	2	75.8	78.4	83.8	66.2	82.4	38.0
Grass artificially dried at exhaust temperature of—							
250° F	3	76.6	76.0	82.4	72.0	83.2	41.4
300° F	3	76.4	75.5	81.9	77.6	82.0	46.8
350° F	3	78.1	75.6	84.5	76.8	83.6	49.8
400° F	3	70.0	59.2	80.9	81.8	76.2	37.3

The digestibility for the various constituents of the sun-cured grass and of that dried at 250°, 300°, and 350° F. showed no significant variation. The digestibility of the constituents in these rations was high, the average being 76 percent for dry matter, 76 for crude protein, 83 for crude fiber, 73 for ether extract, 83 for nitrogen-free extract, and 44 for ash. The sun-cured grass was as efficiently digested as the grass dried by artificial means.

When the temperature of drying was increased to a high level (i. e., the exhaust-gas temperature being 400° F.) for the purpose of securing maximum evaporation of moisture, a lowering of the digestibility of certain of the feed constituents resulted. The apparent digestibility of the constituents of pasture herbage dried at 250°, 300°, and 350° was as high as similar grass dried by 18 hours of exposure in the sunlight. The apparent digestibility of the crude protein of the grass dried at 400° was depressed since it was only 78.3 percent of that of the grass dried at 350°. The apparent digestibility of the dry matter and nitrogen-free extract was also somewhat depressed, being practically the same as the green grass. These findings are in accord with results reported by Watson and Ferguson (*18*) who noted a depression of the digestibility of the protein of grass at an outlet temperature of 200° C. (392° F.).

The apparent digestibility of the various nutrients when the sheep were receiving the green grass was not so high as when they were receiving the dried material. The reasons for this are not entirely apparent. The consumption of dry matter when the sheep were receiving green grass was approximately 15 percent greater than when they were on the dried-grass ration, which may account for a lower utilization of feed nutrients.

NITROGEN, CALCIUM, AND PHOSPHORUS BALANCES

The data pertaining to the nitrogen, calcium, and phosphorus balances of sheep receiving the rations of green, sun-cured, and artificially dehydrated grass are given in table 7. The balance of nitrogen in each case was positive, indicating that the wethers were assimilating considerable of that constituent. Except in the case of the grass dried at 400° F., there was from 2½ to 3 times as much nitrogen recovered in the urine as in the feces indicating that the protein was digested, absorbed, and a large part of the nitrogen was eliminated.

TABLE 7.—*Grams of nitrogen, calcium, and phosphorus ingested, voided, and retained by sheep during 14-day metabolism trials with different rations*

GREEN GRASS, NO TREATMENT

Sheep no.	Nitrogen					Calcium					Phosphorus				
	Ingested	Voided			Retained	Ingested	Voided			Retained	Ingested	Voided			Retained
		Feces	Urine	Total			Feces	Urine	Total			Feces	Urine	Total	
Sheep 1	490.5	125.0	308.1	433.1	57.4	142.5	123.2	3.6	126.8	15.7	90.6	72.6	0.4	72.9	17.7
Sheep 2	527.4	158.4	326.8	485.2	42.3	153.2	155.6	1.6	157.3	[1] 4.1	97.4	89.4	.4	89.7	7.7
Sheep 3	426.1	114.6	283.6	398.3	27.8	123.8	112.8	2.8	115.6	8.2	78.7	67.1	.2	67.4	11.4
Average	478.0	132.7	306.2	438.8	39.2	139.8	130.5	2.7	133.2	6.6	88.9	76.3	.3	76.7	12.3
SUN-CURED GRASS, EXPOSED 18 HOURS															
Sheep 1	466.0	100.9	297.8	398.7	67.3	113.3	99.2	0.2	99.5	13.8	100.2	74.6	0.2	74.8	25.4
Sheep 2	466.0	100.1	308.1	408.2	57.8	113.3	102.3	1.0	103.3	10.0	100.2	74.7	.2	75.0	25.2
Average	466.0	100.5	302.9	403.4	62.6	113.3	100.8	.6	101.4	12.0	100.2	74.4	.2	74.9	25.3
GRASS ARTIFICIALLY DRIED AT 250° F.															
Sheep 1	434.7	107.1	271.0	378.1	56.6	113.2	96.2	0.6	97.4	15.8	80.1	67.6	0.2	67.8	12.3
Sheep 2	434.7	97.9	287.1	385.0	49.6	113.2	98.6	.4	99.0	14.2	80.1	67.6	.2	67.8	12.3
Sheep 4	434.7	107.5	263.8	371.4	63.4	113.2	100.1	.5	100.6	12.6	80.1	71.4	.2	71.6	8.5
Average	434.7	104.2	274.0	378.2	56.5	113.2	98.5	.5	99.0	14.2	80.1	68.9	.2	69.1	11.0
GRASS ARTIFICIALLY DRIED AT 300° F.															
Sheep 1	442.4	109.0	265.4	374.2	68.2	118.7	98.7	0.4	99.0	19.7	86.4	68.7	0.2	68.7	17.6
Sheep 2	442.4	105.4	278.4	384.7	57.7	118.7	99.9	.4	100.1	18.6	86.4	66.7	.2	66.8	19.6
Sheep 4	442.4	111.6	275.0	386.6	55.8	118.7	100.1	.3	100.4	18.3	86.4	72.3	.2	72.5	13.9
Average	442.4	108.6	272.9	371.5	60.9	118.7	99.5	.4	99.9	18.8	86.4	69.2	.2	69.4	17.0
GRASS ARTIFICIALLY DRIED AT 350° F.															
Sheep 1	414.8	97.6	228.7	326.3	88.5	95.5	74.8	0.3	75.1	20.4	73.7	51.6	0.1	51.7	22.0
Sheep 2	452.5	111.2	253.9	365.1	87.4	104.2	90.0	.6	90.6	13.6	80.4	61.8	.1	62.1	18.2
Sheep 3	452.5	114.0	263.4	377.3	75.2	104.2	91.5	.4	92.0	12.2	80.4	63.1	.1	63.2	17.2
Average	439.9	107.6	248.6	356.2	83.8	101.3	85.5	.4	85.1	15.4	78.2	58.8	.1	59.0	19.2
GRASS ARTIFICIALLY DRIED AT 400° F.															
Sheep 1	403.9	169.2	171.8	341.1	62.8	96.6	93.0	0.2	93.2	3.4	81.8	66.7	0.2	66.8	14.9
Sheep 2	441.4	178.2	202.9	381.1	60.4	105.6	102.0	.2	102.2	3.4	89.4	71.3	.2	71.5	17.9
Sheep 4	441.4	177.7	218.6	396.3	45.1	105.6	104.7	.2	105.0	.6	89.4	75.5	.2	75.7	13.7
Average	428.9	175.0	197.8	372.8	56.1	102.6	99.9	.2	100.1	2.5	86.8	71.2	.2	71.3	15.5

[1] This was a minus quantity.

The percentage of nitrogen retained was small and varied considerably with the feeding of the different grass rations. The lowest retention of nitrogen occurred in the experiment with green grass, the average being about 8 percent. The nitrogen recovered in the feces (table 7) was somewhat higher during this trial than on subsequent experiments except for the grass dried at 400° F. where there was a marked depression of the digestibility of the protein. The highest retention of nitrogen occurred when the sheep were receiving the grass dehydrated at 350°. During this trial 19 percent of the ingested nitrogen was retained. There was little variation in the nitrogen balances of sheep when on the rations of sun-cured grass as compared with dehydrated grass dried at either 250°, 300°, or 400°.

The sheep maintained positive calcium balances during each trial, with the exception of sheep no. 2 when it was receiving green grass. This animal failed to utilize any of the calcium in the green grass. There were decided variations in the utilization of calcium by the three sheep during this experiment. This variation may be accounted for by the fact that this was the first trial and the sheep had been on an unknown diet in the stockyard for some time prior to these studies. Such variations were not encountered in subsequent trials. The average calcium balances were not significantly different when the sheep received rations of sun-cured grass and dehydrated grass dried at 250°, 300°, and 350° F.

The sheep receiving rations of pasture grass dried at 400° F. did not utilize the calcium of the rations as efficiently as when receiving similar herbage dried at lower temperatures or cured in the sun. The average calcium balance for sheep receiving rations of grass dried at 400° F. was +2.5 as compared with a balance of +15.4 when receiving grass dried at 350° and a balance of +12.0 when receiving the sun-cured grass. The average calcium balance for the grass dried at 400° was only 16.2 percent as great as for the grass dried at 350° and 20.8 percent as great as for the sun-cured grass.

There was considerably more phosphorus than calcium retained by the sheep when receiving the green grass. The phosphorus balance was highest when the wethers were receiving the sun-cured grass. Apparently the condition causing the poor utilization of calcium in the grass dried at 400° F. did not affect the availability of the phosphorus. The utilization of phosphorus from this grass was approximately as high as from the other dehydrated samples.

The average daily intake of calcium on the various rations was as follows: Green grass, 10.0 g; sun-cured grass, 8.1 g; artificially dehydrated grass dried at 250° F., 8.1 g; dried at 300°, 8.4 g; at 350°, 7.2 g; and at 400°, 7.3 g. The average daily consumption of phosphorus by the sheep on the rations listed was 6.4, 7.2, 5.7, 6.2, 5.6, and 6.2 g, respectively. According to Fraps (*8*) this amount of calcium and phosphorus is considerably in excess of the needs of the wethers used in this experiment. He states that in order for sheep weighing about 100 pounds to maintain a mineral balance they should receive approximately 2 g calcium and 0.54 g phosphorus in their daily ration. While there is some variation in the calcium and phosphorus content of the various rations, this difference does not seem great enough to cause the wide variation in retention of these constituents. The retention of calcium and phosphorus does not seem to bear any relation to the calcium-phosphorus ratio of the rations. The retention of calcium

and phosphorus during the first two trials, which included rations of green and sun-cured grass, may have been influenced by the previous unknown diet of the sheep. While 14-day mineral-balance trials do not give as definite results as longer trials, it does not seem probable that possible deficiencies in pre-experimental rations could have carried through to the trial with the grass dried at 400° F. which followed 16 weeks of feeding on similar rations of high calcium or phosphorus content. It would seem, therefore, that artificial drying of the grass at 400° definitely lowered the calcium retention.

COLOR DETERMINATIONS

A comparison of the color of the different rations of the dried pasture grass is given in table 8. Conversion factors for measuring the percentage of natural color of pasture herbage have not been developed and it is therefore necessary to use those established for alfalfa hay to make the comparison. It is thought that the conversion factors for alfalfa are somewhat high for pasture herbage. The percentage of natural color was highest in the grass that was dried in the sun. This sample of grass was spread thinly on a canvas and exposed to the sunlight. It was taken in at night so was not wet by either rain or dew. This indicates that exposure to moisture is probably a more important factor in causing loss of color than exposure to light. It is probable that grass cured under these conditions would show a higher degree of natural color than ordinary field-cured hay. There were only slight differences between the percentage of natural color of the sun-cured grass and that of grass dried at 250° and 300° F. The color of the dehydrated samples, however, might have been higher had they been dried immediately after cutting, rather than allowed to wilt overnight. The percentage of natural color of the grass dried at 400° was only 76.3 percent of that dried at 250°. The importance of providing forage of a high natural color is indicated by Graves (*9*) who reports a relation between the green color and the assimilable carotene of the feed as well as the yellow color of butterfat produced from the feed.

TABLE 8.—*Relation of temperature of drying to the percentage of natural color of artificially dried pasture grass*

Treatment of grass	Average Munsell hue reading	Percentage of natural color	Grade in terms of alfalfa
Sun-cured grass, exposed on canvas 18 hours	9.50	78	Extra green.
Grass artificially dried at exhaust temperature of—			
250° F	9.22	76	Do.
300° F	8.81	74	Grade 1.
350° F	7.28	65	Do.
400° F	6.15	58	Grade 2.

SUMMARY AND CONCLUSIONS

Six digestive and mineral-balance trials were conducted to determine the effect of the temperature of artificial drying on the apparent digestibility and availability of the feed nutrients in 3-week-old mixed pasture grass. The grass mixture contained about 40 percent of English ryegrass, 40 percent of Italian ryegrass, and 20 percent of white

clover. Exclusive rations of green grass, sun-cured grass, and artificially dehydrated grass dried at exhaust-gas temperatures of 250°, 300°, 350°, and 400° F. were fed to yearling wethers for periods of 21 days, the first 7 of which were preliminary periods.

The percentage of apparent digestibility of the feed constituents in the rations of sun-cured grass and artificially dehydrated grass dried at 250°, 300°, and 350° F. showed no significant differences, indicating that artificial drying at these temperatures had no deleterious effect upon the availability of the nutrients. The percentage of apparent digestibility of the nutrients in the rations of sun-cured grass and dehydrated grass dried at 250°, 300°, and 350° F. averaged for dry matter, 76; for crude protein, 76; for crude fiber, 83; for ether extract, 73; for nitrogen-free extract, 83; and for ash, 44.

The results of feeding pasture grass artificially dehydrated at 400° F. showed that this temperature of drying had a depressing effect on the apparent digestibility of the crude protein, dry matter, and nitrogen-free extract. There was some evidence of burning of the more leafy portions of the herbage when dehydrated at 400° F.

The apparent digestibility of dry matter, crude protein, crude fiber, and nitrogen-free extract was slightly lower for the green grass than for the dried samples, with the exception of that dried at 400° F.

Positive balances of nitrogen, calcium, and phosphorus were maintained by the sheep receiving the six different rations. There was no apparent relationship between the average nitrogen balance and the temperature of artificial drying.

The average calcium balance for the grass dried at 400° F. was very low as compared with the balance for the other dried samples, since it was only 16 percent of the balance obtained for the grass dried at 350° F. The factor causing the low calcium balance for the grass dried at 400° did not act accordingly on the phosphorus.

Under the conditions of this experiment, the percentage of natural color of the herbage was highest in the sample cured in the sun. As the exhaust-gas temperature was increased by 50-degree intervals from 250° to 400° F. in drying the different samples there was a definite lowering of the percentage of natural color in the herbage. The percentage of natural color of the herbage dried at an exhaust-gas temperature of 400° was only 76.3 percent of that dried at 250°.

From the standpoint of the feeding value of the dried product, it is apparent that artificial drying at 400° F. is an uneconomical practice with the machine used in this experiment. Probably the most efficient temperature at which to operate this machine is from 300° to 350°. If high temperatures are employed in the commercial drier without running the temperature at the exhaust end as high as those found to be detrimental in this experiment, forage may probably be dried with little or no depressing effect on the digestibility of the nutrients. Undoubtedly the digestibility of the nutrients in herbage is also influenced by the length of time during which it is subjected to relatively high temperatures.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1931. methods of analysis . . . Ed. 3, 593 pp., illus. Washington, D. C.

(2) Barr, H. T.
1931. artificial dehydration of forage crops. Agr. Engin. 12: 243–244, illus.

(3) Barr, H. T.
1933. POWER, LABOR, AND FUEL REQUIREMENTS OF ARTIFICIAL DRIERS. Agr. Engin. 14: 131–132.
(4) Clyde, A. W.
1932. A SEASON'S TEST OF A HAY DRIER. Agr. Engin. 13: 61–63, illus.
(5) ———
1933. NEW DEVELOPMENTS IN HAY DRIERS. Agr. Engin. 14: 127–129, illus.
(6) Forbes, E. B.
1915. A METABOLISM CRATE FOR SWINE. Ohio Agr. Expt. Sta. Circ. 152, pp. 75–85, illus.
(7) ——— and Grindley, H. S.
1923. ON THE FORMULATION OF METHODS OF EXPERIMENTATION IN ANIMAL PRODUCTION. Bull. Natl. Research Council 6, pt. 2, no. 33, 54 pp.
(8) Fraps, G. S.
1918. MINERAL REQUIREMENTS OF SHEEP. Tex. Agr. Expt. Sta. Bull. 232, 20 pp., illus.
(9) Graves, R. R.
1933. BREEDING, FEEDING, AND MANAGEMENT INVESTIGATIONS. U. S. Dept. Agr., Rept. Chief Bur. Dairy Indus. 1933: 6–13.
(10) Grunder, M. S.
1933. YIELD AND CHEMICAL COMPOSITION OF CERTAIN PASTURE CROPS, FERTILIZED AND UNFERTILIZED. Jour. Agr. Research 46: 375–386.
(11) Hart, E. B., Kline, O. L., and Humphrey, G. C.
1932. THE EFFECT OF ARTIFICIAL DRYING ON THE AVAILABILITY OF THE NUTRIENTS IN ALFALFA. Jour. Agr. Research 45: 507–511.
(12) Henry, W. A., and Morrison, F. B.
1928. FEEDS AND FEEDING; A HANDBOOK FOR THE STUDENT AND STOCKMAN. Ed. 19, 770 pp., illus. Ithaca, N. Y.
(13) Hodgson, R. E., Grunder, M. S., Knott, J. C., and Ellington, E. V.
1934. A COMPARISON OF ROTATIONAL AND CONTINUOUS GRAZING PASTURES IN WESTERN WASHINGTON. Wash. Agr. Expt. Sta. Bull. 294, 36 pp., illus.
(14) ——— and Knott, J. C.
1932. APPARENT DIGESTIBILITY OF, AND NITROGEN, CALCIUM, AND PHOSPHORUS BALANCE OF DAIRY HEIFERS ON, ARTIFICIALLY DRIED HERBAGE. Jour. Agr. Research 45: 557–563.
(15) Honcamp, F.
1915. VERGLEICHENDE UNTERSUCHUNGEN ÜBER DIE ZUSAMMENSETZUNG UND VERDAULICHKEIT VON FRISCHEN GRAS, NATURLICH GEWORBENEM UND DURCH KÜNSTLICHE TROCKNUNG GEWONNENEM HEU. Landw. Versuchs. Sta. 86: [215]–276.
(16) Newlander, J. A., and Jones, C. H.
1932. THE DIGESTIBILITY OF ARTIFICIALLY DRIED GRASS. Vt. Agr. Expt. Sta. Bull. 348, 20 pp.
(17) Nickerson, D.
1920. A METHOD FOR DETERMINING THE COLOR OF AGRICULTURAL PRODUCTS. U. S. Dept. Agr. Tech. Bull. 154, 32 pp., illus.
(18) Watson, S. J., and Ferguson, W. S.
1932. INVESTIGATIONS INTO THE INTENSIVE SYSTEM OF GRASSLAND MANAGEMENT. VIII. THE COMPARATIVE DIGESTIBILITY AND FEEDING VALUE OF FRESH AND ARTIFICIALLY DRIED GRASS. Jour. Agr. Sci. [England] 22: [235]–246.
(19) ——— and Ferguson, W. S.
1932. INVESTIGATIONS INTO THE INTENSIVE SYSTEMS OF GRASSLAND MANAGEMENT. IX. THE DIGESTIBILITY OF ARTIFICIALLY DRIED HAY. Jour. Agr. Sci. [England] 22: [247]–250.
(20) Woodman, H. E., Bee, J. W., and Griffith, G.
1930. NUTRITIVE VALUE OF PASTURE. V. PASTURE GRASS CONSERVATION: THE INFLUENCE OF ARTIFICIAL DRYING ON THE DIGESTIBILITY OF PASTURE HERBAGE. Jour. Agr. Sci. [England] 20: [53]–62.

THE THERMOPHILIC FERMENTATION OF BEET PULP[1]

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INTRODUCTION

The mechanisms of the dissimilative processes involved in the thermophilic fermentation of complex substances such as beet pulp are not clear, but for practical purposes the fermentation may be considered essentially as the break-down of the various carbohydrate constituents, i. e., pectin, pentosans, cellulose, and residual sugars. The importance of the bacterial decomposition of cellulose has been pointed out by Lymn and Langwell (*14*)[2], Tetrault (*15*), Coolhaas (*2*), Fred, Peterson, and coworkers (*9, 12, 16*), and others. The products of this fermentation are acetic and butyric acids, ethyl alcohol, carbon dioxide, hydrogen, and methane. Lymn and Langwell report wide variations in the yields as a result of manipulative treatment of the cultures. Of almost equal importance, not only industrially but also from an agricultural standpoint, is the fermentation of the hemicelluloses and, of somewhat less importance, of the pectins, both of which occur widely distributed in agricultural wastes. Although the thermophilic fermentation of cellulose has been studied for many years, that of the other important constituents of plant tissues for the most part has been neglected.

The present work on the thermophilic fermentation of beet pulp was undertaken with a view to effecting the utilization of an important agricultural byproduct. The important products of this fermentation are acetic and butyric acids, but hydrogen and carbon dioxide are also formed.

EXPERIMENTAL

MATERIALS AND CULTURES

Two kinds of beet pulp were used in the fermentations. One was the dried cossettes left after the extraction of the sugar at the factory; the other was obtained by adding waste molasses to the cossettes and drying (table 1).

TABLE 1.—*Percentage composition of beet pulps used*

Material	Pentosans	Pectin	Cellulose	Total sugar	Lignin	Ether extract	Crude protein	Ash	Moisture
Commercial beet pulp	16.0	34.0	18.0	3.2	2.5	3.5	9.6	2.7	7.5
Beet pulp plus molasses	11.0	26.5	12.5	17.0	2.2	1.1	19.9	5.4	5.3

Mixed bacterial cultures were obtained from sod-manure compost, garden soil, and stable and sheep manure. Enrichment cultures were

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[2] Reference is made by number (italic) to Literature Cited, p. 172.

made by inoculating small quantities of beet-pulp broth and incubating anaerobically at 60° C. for about 48 hours. All fermentations were carried out anaerobically at 60°. In the later stages of the work the only cultures used were enrichment cultures from garden soil, since the products yielded were practically the same in all cases.

BACTERIOLOGICAL STUDIES

The bacteriological study of the fermentation of plant tissues offers many points of interest and many difficulties. It seemed a remote possibility that the complete fermentation of a complex substance such as beet pulp would be brought about by a single bacterial species. It was soon found impracticable, however, to attempt to use pure cultures. After a few transfers had been made the fermentations became sluggish. Purified pectin in concentrations of 0.5 to 2 percent was used in media which were inoculated with crude cultures and fermented in anaerobic jars. Ammonium chloride was used as a source of nitrogen in some cases; peptone was used in others. A fairly active fermentation resulted in all cases. Similar media were prepared, and adjusted to pH 7.0, and transfers were made from the original fermentations. Varying quantities of the fermenting broth were used as inocula. These fermentations were less vigorous. Repeated transfers led to sluggish fermentations. The same experience was encountered in the fermentation of purified xylan. The second transfer brought about a very slow fermentation.

For the isolation of colonies a beet-pulp yeast-infusion agar was used. About 100 g of beet pulp and 50 g of dried yeast in 1 l of water were heated for 15 minutes in the autoclave. After cooling, the supernatant liquid was decanted. Two grams of dipotassium phosphate and 15 g of agar were added, and the medium was again sterilized in the autoclave. The medium was always heated in the autoclave just before being used. Plates were prepared from suitable dilutions of actively fermenting cultures. These plates were placed in vacuum desiccators with actively fermenting broth in the bottom. The desiccators were evacuated and filled with carbon dioxide, and the process was repeated several times. The evolution of gas from the growing cultures in the bottoms of the desiccators maintained a positive carbon dioxide pressure and effectively prevented the diffusion of air into the vessels. Smooth, translucent colonies grew in 24 to 48 hours.

The colonies were transplanted into flasks of beet-pulp broth made from beet pulp containing 17 percent sugar. In a few of the flasks a fairly active fermentation occurred. Transfers were made from these flasks to sugar-free beet pulp, but the fermentations were very slow and unsatisfactory. None of the colonies isolated was regarded as a pure culture; consequently the cultural characteristics were not determined.

The repeated transfer of cultures from one beet-pulp broth to another had the effect of lessening the vigor of fermentation. More formic acid was found in solution after a few transfers were made, but no definite relationship could be established. The effect of the transfer of cultures is indicated in table 2. The building up of an active culture for this fermentation is of considerable importance and should be given further study, especially for the decomposition of pentosans.

TABLE 2.—*Effect of transferring culture on thermophilic fermentation of beet pulp*[1]

Times transferred	Total acid produced [2]		Formic acid	Times transferred	Total acid produced [2]		Formic acid
	Grams	*Percent*	*Percent*		*Grams*	*Percent*	*Percent*
1	21	52.5	0.0	5	9	22.5	5.0
3	18	45.0	1.0	6	14	35.0	7.5
5	15	37.5	10.0	7	8	20.0	2.0

[1] 40 grams of beet pulp in each sample.
[2] Calculated as acetic acid.

While the bacteriological aspects of this work are of scientific and practical importance, it was necessary for the time being to set them aside and continue with the more practical use of crude cultures for the utilization of various forms of agricultural wastes.

ANALYTICAL METHODS

Proximate analyses of the beet pulp were made for the constituents shown in table 1. The official current methods of the Association of Official Agricultural Chemists (*1*) were used for the determination of sugars, pentosans, crude ether extracts, protein, moisture, and ash. Cellulose was determined by the method of Mehta (*6*). Pectin was extracted from the pulp with 0.5 percent ammonium oxalate, according to the method of Schryver and Haynes (*11*), and determined by the procedure recommended by Nanji, Paton, and Ling (*7*). Lignin was determined by Schorger's method (*10*).

According to Schryver and Haynes (*11*), pectin yields 19.8 percent of furfural. Corrections were made accordingly in the determination of the pentosans.

After the fermentations were complete, the liquors were filtered, and analyses were made on both the liquids and solid residues to determine the unaltered constituents. It is possible that the pectin may not have been completely fermented, yet partly hydrolyzed or altered in such a manner as to escape detection after fermentation. The routine examinations for pectin were made after the fermentation.

The volatile acids produced were steam-distilled from an aliquot of the fermentation liquor which had been adjusted to an approximate pH of 4 with sulphuric acid. The acids were determined by the partition method, as described by Osburn and Werkman (*8*).

Carbon dioxide and hydrogen were determined by collecting the gas over water and analyzing the gas mixture in the usual manner. Various forms of apparatus for the collection of gas were made and studied. For thermophilic work the simple expedient mentioned above was the most efficient.

FERMENTATION STUDIES

OXYGEN REQUIREMENTS

Three series of experiments were set up in order to study the oxygen requirements of the organisms. (1) The quantities of beet pulp given in table 3 were added to 1,500 cc of water. Five grams of ammonium chloride and two grams of dipotassium phosphate were added. After inoculation the flasks were aerated for the time indicated. (2) The flasks were stoppered loosely with cotton and shaken

several times a day. (3) In the third series, various kinds of anaerobic apparatus were constructed and used, in some of which elaborate precautions were taken to exclude air. Time and convenience considered, the most efficient was a flask of suitable size with a rubber stopper carrying 2 delivery tubes, 1 of which dipped into mercury. After the medium was inoculated, oxygen-free nitrogen was blown through the flask, and the second delivery tube was sealed. The media were boiled immediately before inoculation to remove air. The data in table 3 indicate that strictly anaerobic conditions result in more complete decomposition of the beet pulp.

TABLE 3.—*Effect of oxygen tension on the thermophilic fermentation of beet pulp*[1]

Duration of experiment (days)	Total acid produced [2]	Acids present		
		Acetic	Propionic	Butyric
	Grams	*Percent*	*Percent*	*Percent*
AERATED CULTURE				
20	1.40	(3)	(3)	(3)
10	1.10	(3)	(3)	(3)
COTTON-STOPPERED FLASK				
10	12.56	86.0	0	14.0
8	10.37	81.5	0	18.5
15	14.75	79.5	0	20.5
ANAEROBIC CULTURE				
10	13.14	57.5	33.8	8.5
10	13.90	69.8	23.0	7.2
10	16.20	80.8	0	19.6
10	16.10	70.7	0	29.3

[1] 40 grams of beet pulp in each sample. [2] Calculated as acetic. [3] Not determined.

INFLUENCE OF pH

Anaerobic fermentations were conducted at pH levels of 5.4, 7.2, and 9.0, as nearly as they could be controlled. For the fermentation at pH 5.4, chlorphenol red was used as an indicator. The broth was neutralized as required. The indicator mixtures for the other two fermentations were prepared according to the method of Kolthoff and Menzel (*5*). At pH 7.2 a mixture of 1 part of 0.1-percent bromthymol blue and 1 part of 0.1-percent phenol red was used. The color changed from yellow to green at pH 7.2 and finally to deep violet at 7.6. Owing to the coloration of the medium, the color changes were sometimes difficult to observe, especially if iron salts were present. At pH 9, 1 part of 0.1-percent thymol blue and 3 parts of 0.1-percent phenolphthalein were used. The broth in all cases was neutralized with sodium hydroxide as required.

The data are given in tables 4 and 5. Complete analyses were made before and after fermentation. The periods of fermentation ranged from 4 to 8 days. It is noteworthy that no hydrogen was produced at pH 9.0 and that while the volatile acids were nearly doubled, as compared with those in the fermentation at 5.4, the grams of carbon

dioxide remained nearly constant (table 5). Very little is known concerning the mechanism of the fermentation of complex substances. Free sugars could never be detected, nor could any intermediate compounds be isolated. The products of fermentation account for 78 percent (at pH 5.4) to 85 percent (at pH 9.0) of the carbon fermented. Ehrlich's (*3*) formula for beet pectin was used in making the calculations. According to the method of Johnson, Peterson, and Fred (*4*) for calculating the oxidation-reduction balance of the products of fermentation, there is an excess of oxidized products in each fermentation. At pH 9.0 the oxidation products are much in excess (-0.70 to $+0.25$). The residue of unfermented beet pulp, including the protein and possibly some degradation products of pectin, is far too complex to permit conjecture as to any reduction products unaccounted for, or the nonappearance of hydrogen at pH 9.0.

TABLE 4.—*Results of thermophilic fermentation of beet-pulp constituents at different pH levels*

Constituent	pH 5.4 (100.5 g)			pH 7.2 (101.4 g)			pH 9.0 (102.5 g)		
	Original sample	Fermented		Original sample	Fermented		Original sample	Fermented	
	Grams	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
Total sugars	17.1	17.1	100.0	17.2	17.2	100.0	17.4	17.4	100.0
Pectin	26.6	20.8	78.2	26.9	26.9	100.0	27.3	27.3	100.0
Pentosans	11.1	8.6	77.5	11.2	8.9	79.4	11.3	9.7	85.8
Cellulose	12.6	2.1	16.6	12.7	1.3	10.2	12.8	7.2	56.2
Lignin	2.2	0	0	2.2	0	0	2.3	0	0

TABLE 5.—*Products of thermophilic fermentation of beet pulp at different pH levels*

Product	pH 5.4 (100.5 g)	pH 7.2 (101.4 g)	pH 9.0 (102.5 g)
	Grams	*Grams*	*Grams*
Hydrogen	0.37	0.23	(1)
Carbon dioxide	14.70	14.90	15.60
Total acid	27.30	34.21	42.60
Formic	(2)	.11	.60
Acetic	13.20	16.70	26.40
Propionic	6.60	9.20	12.10
Butyric	7.50	8.20	3.50

[1] None. [2] Trace.

EFFICIENCY OF NEUTRALIZING AGENTS

Calcium carbonate, sodium bicarbonate, and sodium hydroxide were used as neutralizing agents. The quantities of sugar-free pulp shown in table 6 were inoculated with soil-enrichment cultures. An excess of calcium carbonate was added to some of the flasks. Enough sodium bicarbonate to neutralize acetic acid equivalent to half the weight of the sample of beet pulp used was added to each of the flasks used in experiment 2, and the third set was neutralized from time to time with sodium hydroxide. The results given in table 6 indicate that sodium bicarbonate is the most efficient neutralizing agent used. Sodium carbonate would be used in large-scale operations. Sodium hydroxide would probably be equally efficient if the pH value could be more closely controlled. The inefficiency of calcium carbonate is probably

due to the low pH. The pH remained between 4 and 5 during the experiment. Stirring was purposely avoided.

TABLE 6.—*Efficiency of various neutralizing agents as evidenced by acid production in the fermentation of beet pulp* [1]

Neutralizing agent	Acid produced [2]		Neutralizing agent	Acid produced [2]	
	Grams	*Percent*		*Grams*	*Percent*
$CaCO_3$	6.0	15.0	$NaHCO_3$	15.8	39.5
$CaCO_3$	6.2	15.5	NaOH	10.0	25.0
$NaHCO_3$	16.0	40.0	NaOH	11.5	28.7

[1] 40 grams of sugar-free beet pulp in each sample.
[2] Calculated as acetic.

FERMENTATION OF THE DIFFERENT CONSTITUENTS OF BEET PULP

A series of fermentations was conducted to determine further the optimum conditions. Complete proximate analyses were made after fermentation in order to determine the unfermented components of the pulp. Thirty grams of sugar-free beet pulp, 5 g of ammonium chloride, and 2 g of dipotassium phosphate were added to 1,500 cc of water in 2-l flasks. The flasks were inoculated with enrichment cultures from soil. The grams of each component in the sample and the percentage of each component fermented are given in table 7. The yields and compositions of the acids are given in table 8.

TABLE 7.—*Fermentation of beet-pulp constituents under different experimental conditions* [1] [2]

Constituent	Original sample	Fermented in—					
		Flask 1	Flask 2	Flask 3	Flask 4	Flask 5	Flask 6
	Grams	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Pentosans	4.62	87.0	87.5	70.0	96.0	86.5	78.0
Pectin	10.02	58.5	75.0	62.0	58.0	74.0	59.0
Total sugars	.70	100.0	100.0	100.0	100.0	100.0	100.0
Cellulose	5.37	82.5	84.5	6.5	88.0	89.0	30.4
Lignin	.67	0	0	0	0	0	0

[1] 30 grams of pulp in each sample.
[2] Hydrogen gas was passed through flasks 1, 2, and 3. 15 g of sodium bicarbonate was added to flasks 1 and 2 at the time of inoculation; flask 3 was neutralized with sodium hydroxide (to phenolphthalein) as required. Carbon dioxide was passed through flasks 4, 5, and 6. 15 g of sodium bicarbonate was added to flasks 4 and 5; flask 6 was neutralized with sodium hydroxide as required.

TABLE 8.—*Acids produced by fermentation of beet-pulp constituents under the different conditions shown in table 7*

Flask no.	Total acid produced	Acids present [1]			Flask no.	Total acid produced	Acids present [1]		
		Formic	Acetic	Butyric			Formic	Acetic	Butyric
	Grams	*Percent*	*Percent*	*Percent*		*Grams*	*Percent*	*Percent*	*Percent*
1	11.1	0	86	14	4	13.1	0	85	15
2	12.0	0	76	24	5	14.7	0	86	14
3	7.1	27	60	13	6	7.5	0	85	15

[1] No propionic acid produced.

The greatest effect is to be noted in the fermentation of cellulose. In the presence of the sodium bicarbonate, upwards of 80 percent of the cellulose was fermented; whereas in the other flasks only small percentages were fermented. It is possible that a strain of specific cellulose-fermenting organisms is present in the crude cultures and that conditions for their growth in flasks 3 and 6 were unfavorable. A pure culture of cellulose-fermenting organisms did not ferment beet pulp. The pure culture of Snieszko (*13*), Peterson, and Fred,[3] while active in a cellulose medium, failed entirely to ferment beet pulp.

Lignin was not altered during the period of the fermentation. Apparently pectin is somewhat resistant to fermentation, only about 75 percent breaking down under the most favorable conditions.

Pure cellulose is readily fermented when it is added to beet pulp and inoculated with crude cultures from the soil. Ten grams of beet pulp and five grams of filter paper in 800 cc of water were inoculated with 25 cc of a soil-enrichment culture. The cellulose was entirely fermented at the end of 7 days. The yield of acids was equivalent to 60 percent of the combined weight of pulp and filter paper. The possibilities of the utilization of mixed substrates are being further investigated in this laboratory.

Fermentations conducted under optimum conditions as determined by previous experiments gave yields of acids ranging from 35 to 53 percent of the weight of the beet pulp. The higher yields were obtained from the pulp containing about 17 percent total sugars. Typical results are given in table 9.

TABLE 9.—*Results of thermophilic fermentation of beet pulp with soil-enrichment cultures*

Pulp in sample (grams)	Total acid produced [1]		Acids present		
			Formic	Acetic	Butyric
	Grams	*Percent*	*Percent*	*Percent*	*Percent*
500	267	53.4	0.0	91.0	9.0
500	268	53.6	.0	91.0	9.0
500	250	50.0	.0	89.5	10.5
50	26	52.0	3.0	86.5	10.5
40 [2]	18	45.0	2.0	85.0	13.0
40 [2]	16	40.0	.0	81.5	18.5
40 [2]	14	35.0	.0	85.0	15.0

[1] Calculated as acetic acid. [2] Sugar-free beet pulp.

SUMMARY

The thermophilic fermentation of beet pulp by soil-enrichment cultures has been found to be most efficient at pH 9.0 under strictly anaerobic conditions.

Acetic and butyric acids, and occasionally propionic acid, were formed. The maximum yield was 53 percent of the weight of the beet pulp.

The cellulose, pectin, and pentosans of beet pulp were fermented at about the same rates. Lignin was unaltered.

Repeated transfers of the crude cultures led to sluggish fermentation.

[3] Kindly furnished by Dr. Fred.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1930. official and tentative methods of analysis ... Ed. 3, 593 pp., illus. Washington, D. C.
(2) Coolhaas, C.
1928. zur kenntnis der dissimilation fettsaurer salze und kohlenhydrate durch thermophile bakterien. iii. abhandlung: die dissimilation von zellulose durch thermophile bakterien. Centbl. Bakt. [etc.] (II) 76: 38–44, illus.
(3) Ehrlich, F.
1927. neue untersuchungen über pektinstoffe. Ztschr. Angew. Chem. 40: [1305]–1313.
(4) Johnson, M. J., Peterson, W. H., and Fred, E. B.
1931. oxidation and reduction relations between substrate and products in the acetone-butyl alcohol fermentation. Jour. Biol. Chem. 91: 569–591, illus.
(5) Kolthoff, I. M., and Menzel, H.
1928–29. volumetric analysis. Authorized transl. based on German ed., by N. H. Furman. 2 v., illus. New York and London.
(6) Mehta, M. M.
1925. biochemical and histological studies of lignification. part i. the nature of lignin: its physiological significance and its estimation in timbers. Biochem. Jour. 19: [958]–978.
(7) Nanji, D. R., Paton, F. J., and Ling, A. R.
1925. decarboxylation of polysaccharide acids: its application to the establishment of the constitution of pectins and to their determination. Jour. Soc. Chem. Indus. Trans. 44: 253T–258T.
(8) Osburn, O. L., and Werkman, C. H.
1931. determination of organic acids. v—application of partition method to quantitative determination of acetic, proprionic, and butyric acids in mixture. Indus. and Engin. Chem., Analyt. Ed. 3: 264–265.
(9) Sarles, W. B., Fred, E. B., and Peterson, W. H.
1932. some factors that influence the formation of products in the thermophilic fermentation of cellulose. Centbl. Bakt. [etc.] (II) 85: 401–415, illus.
(10) Schorger, A. W.
1926. the chemistry of cellulose and wood. 596 pp., illus. New York.
(11) Schryver, S. B., and Haynes, D.
1916. the pectic substances of plants. (preliminary communication.) Biochem. Jour. 10: [539]–547.
(12) Scott, S. W., Fred, E. B., and Peterson, W. H.
1930. products of the thermophilic fermentation of cellulose. Indus. and Engin. Chem. 22: 731–735, illus.
(13) Śnieszko, S.
1933. the isolation of a thermophilic cellulose-fermenting organism. Centbl. Bakt. [etc.] (II) 88: 403–409, illus.
(14) Society of Chemical Industry, London Section.
1923. discussion on the action of bacteria on cellulosic materials. Jour. Soc. Chem. Indus. 42: 279T–287T. (Contribution by A. H. Lymn and H. Langwell, pp. 280T–283T.)
(15) Tetrault, P. A.
1930. the fermentation of cellulose at high temperatures. Centbl. Bakt. [etc.] (II) 81: 28–45, illus.
(16) Viljoen, J. A., Fred, E. B., and Peterson, W. H.
1926. the fermentation of cellulose by thermophilic bacteria. Jour. Agr. Sci. [England] 16: 1–17, illus.

THE ISOLATION AND DISTRIBUTION OF NITROGEN IN DILUTE ALKALI-SOLUBLE PROTEINS OF HEALTHY VALENCIA AND WASHINGTON NAVEL ORANGE FRUITS[1]

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INTRODUCTION

It is generally recognized that the application of nitrogenous fertilizers to the soil has an important bearing on the metabolism of the citrus tree and on the production of normal fruit, and yet the percentage of total nitrogen in the mature fruit is very low. The effect of nitrogenous fertilization on the accumulation of nitrogen in the tissues has not been determined for citrus as it has been for certain other plants.

This paper presents the results of one phase of a general investigation of the nitrogenous constituents of citrus fruits. The investigation includes a study of the effects of fumigation, sprays, storage, and other factors on these constituents. The present paper deals only with the isolation and distribution of nitrogen in the proteins of the solids in the edible portion of healthy orange fruits.

REVIEW OF LITERATURE

Very few papers have been published bearing on the organic forms of nitrogen existing in *Citrus*, and practically no correlation has been made of their function in metabolism.

Smith (*13*)[2] isolated a protein from the edible portion of the orange and very briefly described its general properties. It was found to be soluble in dilute alkali (0.3 percent) and insoluble in water, neutral salts, and weak acids. It was not coagulated by heat in neutral or alkaline solution and it possessed an isoelectric point of pH 4.7.

Nelson, Mottern, and Eddy (*9*) identified nitrogenous compounds such as arginine, asparagine, choline, and stachydrine, and a mixture of monoamino acids in the juice of the Valencia orange.

Saunders (*12*) and Rotha and Saunders (*11*) treated orange-seed meal with various alkali halides in normal concentrations and observed that the various salts extracted the same amounts of nitrogen. They also found that the protein isolated by each of these different salts had the same amount of nitrogen. As a result of their work they concluded that they were dealing with a definite globulin and that this was the only type of protein existing in the seed of the orange.

The proteins which have been found in the edible portion of the orange and which are characterized by being insoluble in neutral solvents but soluble in dilute acids and alkalis (*5*), are not commonly found in fleshy, acid fruits. They usually occur in the endosperms of

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[2] Reference is made by number (italic) to Literature Cited, p. 180.

seeds, as found by Larmour (*8*), and may exist in the cytoplasm of the green tissues, as found by Chibnall (*3*), who isolated such a protein from spinach leaves.

EXPERIMENTAL METHODS

ISOLATION OF THE PROTEIN

The method used in extracting the protein from the Valencia orange fruit was a modification of the method used by Smith (*13*). It was as follows:

Pulp of burred oranges or separator pulp was washed with cold water. "Separator pulp" is the term applied to the suspended solids which have been thrown down by centrifuging the mechanically macerated, fleshy part of the fruit. The washed pulp was then extracted with an equal volume of 95-percent alcohol containing 1-percent NaOH. This material was filtered through cotton cloth. The pulp was discarded and an equal volume of water was added to the filtrate. Acetic acid was added slowly to the filtrate to bring it to pH 4.7, at which point the protein settled out as a flocculent precipitate. The precipitate was washed with distilled water until acid-free. It was then extracted with alcohol (95 percent) to remove color, and finally washed with absolute alcohol and anhydrous ether and dried in a vacuum oven at 40° C.

The protein was then placed in a 0.3-percent NaOH solution and filtered. Much of this material would not dissolve in 0.3-percent alkali. The insoluble residue was treated with 0.3-percent alkali until solution of the protein ceased. The protein was then precipitated at pH 4.7 with acetic acid. The processes of dissolving and precipitating were repeated three times. Finally the precipitated protein was washed with 95-percent alcohol, absolute alcohol, and anhydrous ether, and was dried in a vacuum oven at 40° C. The protein was then in the form of a friable, grayish-white powder.

A more elaborate procedure was required for the extraction of protein from the Washington navel orange. Complications arose owing to the possible existence of two proteins in the pulp. The protein material also appeared to be more firmly associated with the chromophores than in the Valencia orange. The method employed for isolating protein material from the Washington navel orange was as follows:

The pulp was washed with hot water. The washed pulp was extracted three times with 95-percent ethyl alcohol to free the material of pigments. The pulp was then dried in vacuum and subsequently extracted with 5-percent NaOH. The alkali extract was treated with 2 volumes of 95-percent ethyl alcohol and filtered. The protein was precipitated from the filtrate with acetic acid at pH 4.6 to 4.7. After the material had been washed free of acid with distilled water, it was shaken with 95-percent alcohol containing 1-percent NaOH. Some of the precipitated protein was dissolved in this solution and was designated as protein A, while that which remained insoluble in the alcoholic alkali was designated as protein B.

The solution containing protein A was precipitated at pH 4.6 to 4.7 with acetic acid and filtered. The acid was removed from the filtrate by washing with distilled water, the color was removed by using 95-

percent ethyl alcohol, and, finally, the precipitate was washed with anhydrous ether and dried in a vacuum oven at 40° C.

Preliminary tests demonstrated that this product was contaminated with carbohydrates. Further purification was accomplished by dissolving it in a 0.3-percent NaOH solution, filtering, and precipitating the protein by adding dilute acetic acid until the solution reached a pH value of 4.6 to 4.7. The protein was dehydrated with 95-percent ethyl alcohol, absolute alcohol, anhydrous ether, and finally dried in vacuum at 40° C. This procedure was performed a second time. When the protein was precipitated at pH 4.7 and allowed to stand overnight, the supernatant liquid was free from any turbidity.

The insoluble material designated as the protein B fraction contained much carbohydrate material and a gummy substance of a colloidal nature. Distilled water was added to this material, which was already alkaline, until the protein dissolved. The protein was then precipitated at pH 4.7 with acetic acid. After the precipitate had been filtered, the protein was extracted with a 1-percent acetic acid solution until washings were free of carbohydrate. It was next freed of acid with distilled water, and of color with 95-percent ethyl alcohol, and finally washed with anhydrous ethyl ether, and dried in vacuum at 40° C. The total nitrogen of this material was 12.29 percent, corrected for moisture and ash, the latter being 0.95 percent. Since at this time it was impossible to obtain enough material for further experimentation, no attempt was made to determine whether this nitrogen-containing material was similar to the protein already isolated or to another protein having different physical and chemical properties.

CHEMICAL METHODS

The nitrogen fractions recorded in table 1 were determined by slight modifications of the methods of Van Slyke (*14*). The apparatus used in the ammonia nitrogen determination was the same as that described by Cavett (*2*). The latter procedure saved much time and yet retained the accuracy of the original method. The modified method employed by Plimmer and Rosedale (*10*) was used in some of the determinations. According to this method the phosphotungstic acid was removed from the basic filtrate as well as from the basic fraction by the addition of $BaCl_2$. This was done to rid the filtrate of salts that crystallized during the distillation process, and it allowed the filtrate to be reduced to a much smaller known volume. It was found, however, that phosphotungstic acid did not interfere with the amino nitrogen determination of the filtrate. In the amino nitrogen determination, it was desirable that the final volume of the basic filtrate should be as small as possible, since 2-cc aliquots were taken for the determination.

The apparatus used in the arginine determination was that described by Holm (*7*). When samples of protein weighing less than 1 g were used, the modified apparatus of Cavett (*2*) was found very satisfactory. In the determination of the ammonia fraction, the distillate was received in N/14 H_2SO_4, while in the other nitrogen determinations, the ammonia was received in 4-percent H_3BO_3.

TABLE 1.—*Nitrogen distribution of protein of the Valencia and Washington navel orange pulp, expressed as percentage of the total nitrogen*

Nitrogen containing compound or form of nitrogen	Valencia			Washington navel		
	A	B	Average	A	B	Average
Ammonia	5.05	5.38	5.21	7.15	6.87	7.01
Acid insoluble	1.35	1.30	1.33	.80	.77	.79
Acid soluble	1.48	1.40	1.44	.88	.77	.82
Total humin	2.83	2.70	2.77	1.68	1.54	1.61
Arginine	12.14	12.28	12.21	12.55	12.66	12.61
Histidine	2.55	2.64	2.60	2.58	2.47	2.52
Cystine						
Lysine	10.73	10.78	10.75	10.48	10.40	10.44
Total basic	25.42	25.70	25.56	25.61	25.53	25.57
Amino	58.50	58.91	58.70	58.75	59.25	59.00
Nonamino	7.87	7.46	7.67	7.38	7.24	7.31
Total nonbasic	66.37	66.37	66.37	66.13	66.49	66.31
Total nitrogen	99.67	100.15	99.91	100.57	100.43	100.50

The modification of Larmour (*8*) was used in the precipitation of the basic fraction. Instead of the solution being heated for 1 hour after the bases had been precipitated with phosphotungstic acid, the two separate solutions, that is, the basic and the phosphotungstic acid solutions, were heated to boiling and poured together.

THE ANALYSIS OF THE PROTEINS

The percentage of total nitrogen of the proteins from the two kinds of oranges are recorded in table 2, corrected for moisture and ash. The figures for the ash content of both proteins are not all that could be desired, but to free the protein of all of its ash constituents would require many precipitations which would result in great loss of protein. The proteins were precipitated at pH 4.6 to 4.7, as shown in the table. They also had a minimum solubility at these pH values when precipitated from a solution of 0.3-percent NaOH by the addition of dilute acetic acid.

TABLE 2.—*Comparative analyses (percentage) of Valencia and Washington naval orange protein*

Constituents	Valencia	Washington navel	Constituents	Valencia	Washington navel
Nitrogen	15.12	15.37	Ash	1.05	0.70
Moisture	7.32	8.70	pH [1]	4.70	4.60–4.70

[1] Isoelectrically precipitated.

The data recorded in table 1 show the distribution of nitrogen in the proteins from the Valencia and Washington navel orange pulp, respectively. The proteins of the Valencia orange possessed more humin nitrogen than the protein of the Washington navel orange, which is probably due to contamination with carbohydrates which

furnish an adequate amount of aldehyde for humin formation. The acid hydrolysates of these proteins were dark in color, which is characteristic of hydrolysates high in humin nitrogen.

The ammonia nitrogen was somewhat higher in the navel orange protein. This value of a protein analysis is supposedly derived from the amide nitrogen of the monoamino dicarboxylic acids and also from deaminization of some of the α-amino groups. Gortner and Holm (*6*) have shown that the ammonia derived from the deaminization of the amino groups varies with the length of hydrolysis. They found that on prolonged hydrolysis appreciable deaminization of the amino groups may occur, giving rise to an abnormally high value for the true amide nitrogen.

The values of the basic nitrogen of the two proteins are the same, within the limits of experimental error. This value represents the nitrogen precipitated with phosphotungstic acid, and is a measure of the arginine, cystine, histidine, and lysine content. The calculations of the percentage of these amino acids were made according to the method of Van Slyke (*14*).

The nonbasic nitrogen which represents the monoamino monocarboxylic acids and monoamino dicarboxylic acids is 66 percent of the total nitrogen. More than 58 percent of the total nitrogen occurs in the monobasic fraction in the amino form, while more than 7 percent occurs as nonamino nitrogen.

An effort was made to determine quantitatively the amount of pentose in the protein material for the purpose of ascertaining whether the pentose is precipitated with the protein as an impurity or whether it is an integral part of the protein molecule. This was done by determining the yield of furfural from 0.5 g of orange protein distilled according to the official method (*1*). The distilled furfural (or methyl furfural) was subsequently precipitated with phloroglucinol. The amount of furfural obtained was 4.7 mg, which, in all probability, was derived from the arabinose and galacturonic acid of pectin. The decarboxylation of galacturonic acid in the presence of the 12-percent HCl yields arabinose which, in turn, would produce furfural. Hence, it is very probable, as Smith (*13*) has noted, that the pentose reaction was derived from the pectin accompanying the protein in the precipitation process and that it is not an integral part of the protein molecule. It should be mentioned here that the phloroglucide precipitate was nearly all soluble in 95-percent ethyl alcohol. It may be that the presence of this alcohol-soluble phloroglucide (*4*) was due to the formation of methyl furfural from the hydrolysis of pectin.

It is known that when pectin is treated with dilute NaOH, a soluble sodium pectate is produced, and when the pectate is acidified with HCl a gelatinous, insoluble pectic acid is formed. This is probably the reason why it is so difficult to free the protein of pentoses, or furfural-yielding substances, by repeated precipitation from dilute NaOH, for during the process the pectic acid is also partially precipitated.

A previous investigation (*13*) has shown that this protein gives the biuret reaction, yielding the pink color characteristic of proteose and peptone. The alkaline solution of orange protein possessed no turbidity and therefore showed no Tyndal cone. When a 1-percent solution of the protein, dissolved in 0.3-percent NaOH, was pla ce

in a cellophane bag and dialyzed against distilled water for 48 hours, no protein diffused through the bag. Some of the sodium came through the membrane, but enough was retained in the bag to prevent the precipitation of the protein during the 48-hour dialysis.

Experiments were performed to determine the effect of heat on the precipitation of the protein from a 0.3-percent NaOH solution. Fifty cubic centimeters of a 1-percent navel orange protein solution was brought to the boiling point in 4 minutes and maintained at a boiling temperature for 5 minutes, cooled to room temperature, and then precipitated at pH 4.7 by the addition of acetic acid. These experiments were repeated except that the boiling time was lengthened to 10 and 20 minutes, respectively. In each case a noticeable difference occurred in the rate of precipitation from the alkaline solution. In the case of the solutions boiled for 20 minutes, some of the protein was precipitated at pH 4.7, but much of it remained in dispersion and the supernatant liquid did not clear as it had done in the control experiments. This demonstrated that the very short period of heating of the protein solution at boiling temperature in dilute alkali produced enough hydrolysis or cleavage in the molecule to alter its precipitation at the isoelectric point.

DISCUSSION

Table 3 records the amide, humin, basic, and nonbasic nitrogen of proteins soluble in dilute alkali from various sources, as reported by other investigators and the writers. An inspection of table 3 shows a similarity between the nitrogen fractions of the proteins of Valencia and navel orange fruits and those of other plant tissues. It is not claimed however that all proteins soluble in dilute alkali would yield nitrogen fractions similar to those reported in the table, for existing evidence (*8*) shows otherwise. It is sufficient to say that the values demonstrate the existence of a class of proteins in plant tissues, soluble in dilute alkali, which is characterized by a relatively high content of both basic nitrogen (25 percent ±) and of amide nitrogen (5 to 10 percent).

TABLE 3.—*Comparison of the amide, humin, basic, and nonbasic nitrogen of the proteins soluble in dilute alkali from various plant sources*

Nitrogen	Percentage of protein from indicated sources—					
	Spinach leaves [1]	Rice [2]	Rye [2]	Spelt [2]	Valencia orange fruit	Washington navel orange fruit
Amide	6.94	8.07	7.14	8.06	5.21	7.01
Humin	2.06	1.95	4.30	5.02	2.77	1.61
Basic	26.59	27.59	25.79	24.44	25.56	25.57
Nonbasic	64.41	62.52	62.50	62.07	66.37	66.31

[1] Data from Chibnall (*5*). [2] Data from Larmour (*8*).

As Smith (*13*) has noted, the proteins of the orange exist in the solid state. This is manifested by the fact that these proteins are soluble in dilute alkali and are precipitated at pH 4.7, while the pH of the juice is approximately 3.5.

Further research is necessary to establish the factors affecting the equilibrium which in all probability must exist between the proteins in the solid state in the fruit and other simpler nitrogenous compounds which are known to exist in solution. A previous investigation (*9*) has identified in the juice of Valencia oranges such soluble nitrogenous compounds as arginine, asparagine, choline, and stachydrine, and a mixture of monoamino acids. It is very probable that the amino acids are synthesized in the leaves, transported to the fruit, and later synthesized to protein in the fruit. When the nitrogen bases are considered, little is known of their direct function in plant metabolism, and it has never been determined whether these compounds are directly active in protein synthesis or are only metabolic products formed by the breaking down of amino acids in the plant.

SUMMARY AND CONCLUSIONS

Detailed methods have been described for the isolation and purification of the dilute alkali-soluble proteins from the Valencia and Washington navel orange pulp, respectively. The distribution of the nitrogen of these proteins has been determined according to the method of Van Slyke (*14*), as modified by various other workers.

The experimental results recorded in this paper appear to warrant the following conclusions:

The protein from the Valencia orange pulp has a nitrogen content of 15.12 percent and that from the Washington navel orange pulp, 15.37 percent, both being corrected for moisture and ash.

The basic amino acid fraction and the nonbasic fraction of the two proteins are practically the same.

Protein from the Washington navel orange has a slightly higher percentage of ammonia nitrogen than that from the Valencia orange, but the protein from the latter has a higher percentage of humin nitrogen. It is possible that impurities may account for the differences in the humin values.

Both of these proteins were precipitated in pH 4.6 to 4.7. This is the pH range of minimum solubility as determined by the amount of protein remaining in solution after precipitating the protein at these pH values.

After repeated precipitation from a 0.3-percent NaOH solution, the protein contained carbohydrate material. When the protein was distilled with 12-percent HCl, furfural was produced, which in turn yielded an alcohol-soluble phloroglucide when precipitated with phloroglucinol.

When a dilute aklali solution (0.3-percent NaOH) of Washington navel orange protein was heated to the boiling temperature for 5, 10, and 20 minutes, respectively, a difference occurred in the rate of precipitation of the protein at its isoelectric point. Heat did not coagulate the protein.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1930. official and tentative methods of analysis . . . Ed. 3, 593 pp., illus. Washington, D. C.

(2) Cavett, J. W.
1932. a modification of the van slyke nitrogen distribution method. Jour. Biol. Chem. 95: 335–343, illus.

(3) Chibnall, A. C.
1924. spinacin, a new protein from spinach leaves. Jour. Biol. Chem. 61: 303–308.

(4) Dore, W. H.
1926. the composition of pectin: a preliminary report on the determination of galacturonic acid in pectin. Jour. Amer. Chem. Soc. 48: 232–236.

(5) Gortner, R. A.
1929. outlines of biochemistry, the organic chemistry and the physico-chemical reactions of biologically important compounds and systems, 793 pp., illus. New York and London.

(6) ——— and Holm, G. E.
1917. the effect of prolonged acid hydrolysis upon the nitrogen distribution of fibrin with especial reference to the ammonia fraction. Jour. Amer. Chem. Soc. 39: 2736–2745, illus.

(7) Holm, G. E.
1920. a modification of the apparatus for the determination of arginine nitrogen by van slyke's method. Jour. Amer. Chem. Soc. 42: 611–612, illus.

(8) Larmour, R. K.
1927. a comparative study of the glutelins of the cereal grains. Jour. Agr. Research 35: 1091–1120.

(9) Nelson, E. K., Mottern, H. H., and Eddy, C. W.
1933. nitrogenous constituents of florida valencia orange juice. Fruit Products Jour. 12: 231–235, 250.

(10) Plimmer, R. H. A., and Rosedale, J. L.
1925. analysis of proteins. v. van slyke's method of determination of nitrogen distribution. Biochem. Jour. 19: [1004]–1014.

(11) Rotha, L. K., and Saunders, F.
1932. studies in proteins. iii. the uniform solubility of the protein fraction of orange seed meal in solutions of various sodium salts. Jour. Amer. Chem. Soc. 54: 342–345.

(12) Saunders, F.
1931. studies in proteins. ii. concerning the uniformity of the protein fraction extracted from orange seed meal salt solutions. Jour. Amer. Chem. Soc. 53: 696–700.

(13) Smith, A. H.
1925. a protein in the edible portion of orange. preliminary paper. Jour. Biol. Chem. 63: 71–73.

(14) Van Slyke, D. D.
1911. the analysis of proteins by determination of the chemical groups characteristic of the different amino-acids. Jour. Biol. Chem. 10: 15–55, illus.

THE VITAMIN A CONTENT OF FIVE VARIETIES OF SWEETPOTATO [1]

By F. L. MacLeod, *associate professor of home economics*, M. R. Armstrong, M. E. Heap, and L. A. Tolbert, *graduate students, Tennessee Agricultural Experiment Station*

INTRODUCTION

Steenbock and Sell (*9*) [2] in 1922 studied three varieties of sweetpotatoes and reported that the amount of vitamin A found in them varied with the depth of color. More recent investigations (*1*, *3*, *5*) have indicated clearly that vitamin A activity varies with the carotene content of plant products. In 1931 Rice and Munsell (*6*) reported the vitamin A content of sweetpotatoes tested by them to be 1,360 Sherman units per pound.

The experiments described in this paper were carried out for the purpose of determining the vitamin A value of different types of sweetpotato (*Ipomoea batatas* (*L.*) *Lam.*) commonly grown in Tennessee. Three of the varieties studied—the Nancy Hall, Yellow Jersey, and Porto Rico—are strongly pigmented, the Porto Rico having the deepest color and the Nancy Hall and Yellow Jersey having about the same depth of color (*4*). The Triumph and Southern Queen are much lighter in color. The sweetpotatoes were grown on the University of Tennessee Farm. They were cured at 85° to 90° F. and stored at 50° to 60°.

The Sherman and Munsell (*8*) method of testing for vitamin A as modified by Sherman and Burtis (*7*) was used in all of the experiments.

The potato was fed raw and was cut in such a way that the animal always obtained a representative cross-section of it.

VITAMIN A CONTENT OF THE NANCY HALL SWEETPOTATO

The studies dealing with the vitamin A value of the Nancy Hall sweetpotato were carried out during 1 year.

The first series of experiments was started immediately after the harvesting of the sweetpotatoes. More variable results were obtained and a lower vitamin A value was found in these experiments than in tests carried out several months after harvesting The feeding of the sweetpotato was continued from early in November through June.

Figure 1 shows the results of the experiments in which the Nancy Hall variety was fed. When 0.025 g was fed daily to each animal 6 days in the week, 8 animals lost an average of 6 g in 8 weeks. When 0.05 g was fed daily, 10 animals made an average gain of 57 g in 8 weeks. Therefore, the quantity of this variety which contains 1 vitamin A unit, that is, the amount which will induce a gain of 25 g in 8 weeks in a standard test rat, is between 0.025 and 0.05 g, or approximately 0.035 g. The Nancy Hall variety contains, therefore, about 30 units of vitamin A per gram, or 13,500 units per pound.

[1] Received for publication Dec. 21, 1934; issued April, 1935. Contribution from the Agricultural Experiment Station and the School of Home Economics of the University of Tennessee.

[2] Reference is made by number (italic) to Literature Cited, p. 186.

VITAMIN A CONTENT OF THE PORTO RICO SWEETPOTATO

The Porto Rico variety was studied at two different times during the year. In series 1 the sweetpotatoes were fed directly after harvesting, while in series 2 they were fed after having been stored for 2 months or more. The results of the experiments of the two series varied too much to be averaged together, and they are therefore presented separately, as series 1 and series 2.

SERIES 1

The results of series 1, in which the Porto Rico sweetpotato was fed directly after harvesting, are shown in figure 2, *A*. The gains made by the different animals receiving the same amount were variable. The results indicated, however, that a unit of vitamin A was contained in somewhat less than 0.05 g of the Porto Rico variety. Four animals each of which received 0.05 g of sweetpotato per day gained an average of 34 g in the 8 weeks of the experimental period, while 4 which received 0.025 g each per day lost an average of 15 g. These results indicate that at this season, directly after harvesting, the Porto Rico variety contained about 20 units of vitamin A per gram, or 9,000 units per pound.

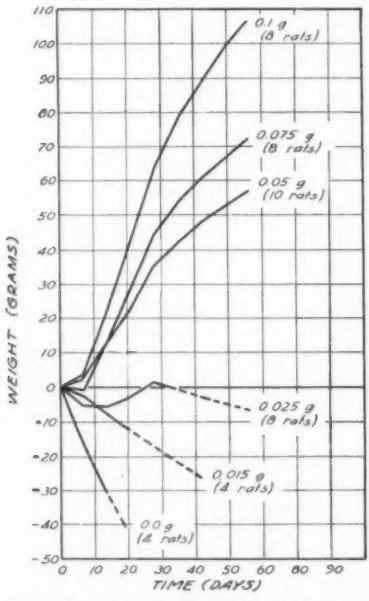

FIGURE 1.—Vitamin A content of the Nancy Hall sweetpotato, showing average gain or loss in weight of rats fed this sweetpotato as their sole source of vitamin A. The amount of sweetpotato fed per rat per day and the number of rats in the group are shown at the end of each curve; broken lines indicate the death of one or more animals.

SERIES 2

The results of the experiments of series 2, in which the Porto Rico variety was fed 2 months or more after harvesting, are given in figure 2, *B*. Seven rats each of which received 0.015 g of sweetpotato per day made an average gain of 37 g in an 8-week period. This was approximately the same gain as that made by the animals of series 1, which received 0.05 g per day. The vitamin A value of the potato had increased about three times, therefore, in the few months after harvesting. Amounts varying from 0.0075 g per rat per day to 0.075 g were fed in this series. The results indicate that the Porto Rico sweetpotato, after storage for a few months, contained at least 65 units of vitamin A per gram, or 29,000 per pound. Fraps and Treichler (*2*) reported 50 units per gram, as the vitamin A value of Porto Rico sweetpotatoes, a figure which compares favorably with that found in series 2.

The marked increase in the vitamin A value of the sweetpotatoes which was observed during the year could not be accounted for by

changed conditions in the laboratory or by any change in the animals. The negative control animals used in the experiments of series 1 lived 23.4 days and lost an average of 40.3 g, while those of series 2 lived 25.0 days and lost an average of 36.0 g. These findings clearly show that the animals of the two series reacted similarly to a diet devoid of vitamin A, and prove that any differences in the results of the two series were due to changes taking place in the sweetpotato during storage.

VITAMIN A CONTENT OF THE YELLOW JERSEY SWEETPOTATO

The Yellow Jersey sweetpotato used in this experiment had a deep yellow flesh, similar in depth of color to the Nancy Hall variety.

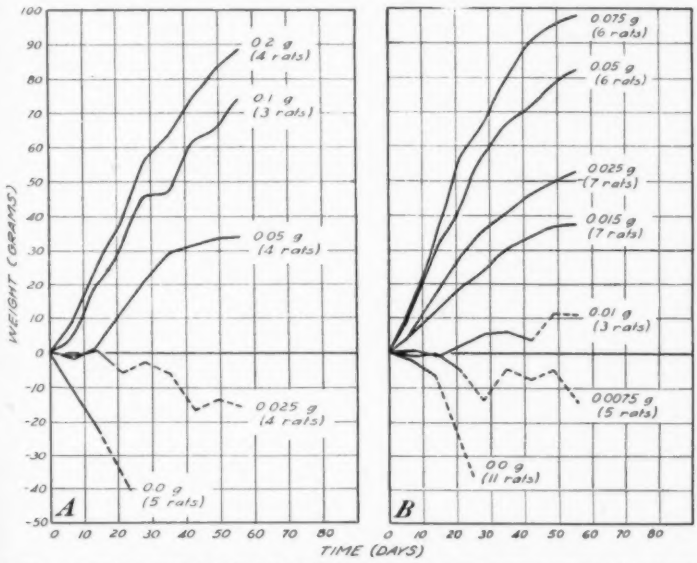

FIGURE 2.—Vitamin A content of the Porto Rico sweetpotato in experiments of series 1 (*A*) and series 2 (*B*), showing average gain or loss in weight of rats fed this sweetpotato as their sole source of vitamin A. The amount of sweetpotato fed per rat per day and the number of rats in the group are shown at the end of each curve; broken lines indicate the death of one or more animals. In series 1 the sweetpotatoes were fed directly after harvesting; in series 2 they were fed several months after harvesting.

The studies reported here were made at two different times of the year and during the same year in which the experiments on the Porto Rico variety were carried out. The experiments of series 1 were performed in the fall, directly after harvesting, and those of series 2 several months later.

SERIES 1

Amounts of Yellow Jersey sweetpotato varying from 0.025 g to 0.2 g per rat per day were fed directly after harvesting. The results are shown in figure 3, *A*. Four rats receiving 0.1 g each per day made an average gain of 10 g in 8 weeks, while three rats receiving 0.2 g each per day made an average gain of 80 g. These results indicate that the Yellow Jersey variety directly after harvest contained less

than 1 vitamin A unit in 0.1 g; that is, less than 10 units per gram or less than 4,500 units per pound.

SERIES 2

In a second series of experiments Yellow Jersey sweetpotato was fed after it had been in storage for several months. The amounts fed varied from 0.015 to 0.1 g per rat per day. The results are shown in figure 3, *B*. When 0.025 g per rat per day was fed to 7 rats, the animals made an average gain of 25 g in the 8 weeks of the experimental period. When 0.05 g per rat per day was fed to 7 rats, the animals made an average gain of 53 g. The sweetpotato at this time, therefore, contained about 40 units of vitamin A per gram, or 18,000 units per pound. The vitamin A potency of the sweetpotato had increased at least four times during storage.

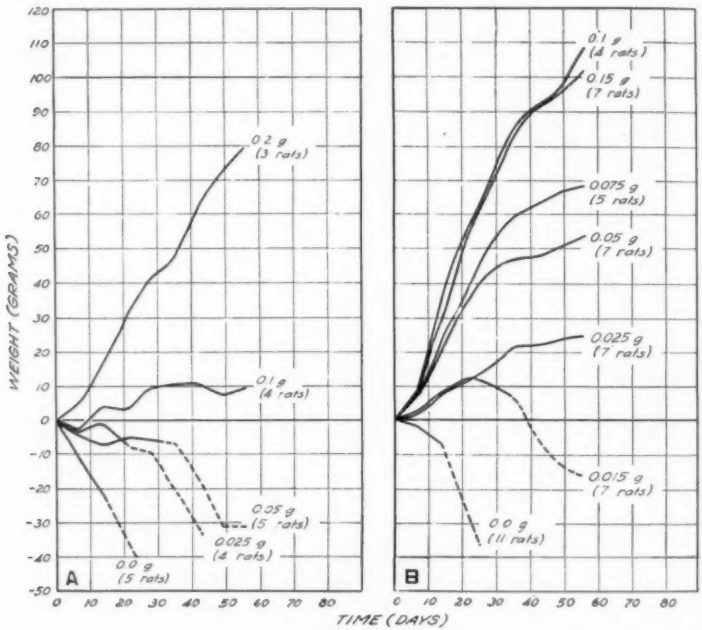

FIGURE 3.—Vitamin A content of the Yellow Jersey sweetpotato in experiments of series 1 (*A*) and series 2 (*B*), showing average gain or loss in weight of rats fed this sweetpotato as their sole source of vitamin A. The amount of sweetpotato fed per rat per day and the number of rats in the group are shown at the end of each curve; broken lines indicate the death of one or more animals. In series 1 the sweetpotatoes were fed directly after harvesting; in series 2 they were fed several months after harvesting.

It is apparent, therefore, that a change takes place in the vitamin A content of sweetpotatoes during storage. Determinations of the water content of different varieties of sweetpotato at different times of the year [3] have shown that the moisture content does not change sufficiently to account for the variation in vitamin A activity. The increase in vitamin A activity in the months after harvesting may

[3] Unpublished data. University of Tennessee.

indicate that the carotene in the sweetpotato is not fully developed when the roots are first harvested, that the carotene has not yet developed into the precursor of vitamin A, or else that this precursor (the carotene responsible for the formation of vitamin A in vivo) is present in a form less available to the animal body when the sweetpotatoes are first harvested than after they have been stored for some time.

VITAMIN A CONTENT OF THE TRIUMPH SWEETPOTATO

The Triumph sweetpotato was first fed directly after harvesting. The levels of feeding chosen, which were the same as those used in testing the Nancy Hall, Yellow Jersey, and Porto Rico varieties, were too small to promote growth. All the ainmals in these tests died before the end of the experimental period and showed typical symptoms of vitamin A deficiency.

Another group of animals were fed after the potatoes had been stored for 2 months or more. Feeding levels of 0.2, 0.4, 0.6, and 0.8 g per rat per day 6 days per week were given. Eight rats receiving 0.2 g per rat per day lost an average of 9.8 g in the 8 weeks experimental period. Eleven rats receiving 0.4 g per rat made an average gain of 21.2 g, while 3 rats which received 0.6 g per day made an average gain of 45.3 g. The Triumph variety contains, therefore, about 2 units of vitamin A per gram, or 900 units per pound. The results are shown in figure 4.

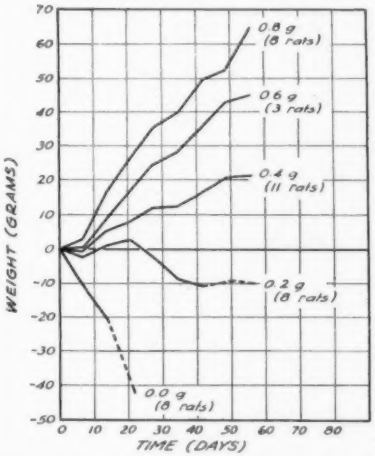

FIGURE 4.—Vitamin A content of the Triumph sweetpotato, showing average gain or loss in weight of rats fed this sweetpotato as their sole source of vitamin A. The amount of sweetpotato fed per rat per day and the number of rats in the group are shown at the end of each curve; broken lines indicate the death of one or more animals.

VITAMIN A CONTENT OF THE SOUTHERN QUEEN SWEETPOTATO

The Southern Queen was also found to be a poorer source of vitamin A than the Nancy Hall, Porto Rico, and Yellow Jersey varieties. The same levels of feeding were used in testing this variety as in the tests with the Triumph variety. It was fed only after storage for 2 months or more. Ten rats which received 0.2 g of the Southern Queen variety per rat per day made an average gain of 21.7 g in the 8 weeks period, while 11 which received 0.4 g made an average gain of 52.3 g.

The Southern Queen variety contained, therefore, about 4 vitamin A units per gram, or 1,800 units per pound. The results are shown in figure 5.

SUMMARY AND CONCLUSIONS

The vitamin A content of five varieties of sweetpotato—Nancy Hall, Porto Rico, Yellow Jersey, Triumph, and Southern Queen was studied.

The Porto Rico and Yellow Jersey types were investigated at two different seasons; first, directly after harvesting in the fall, and again 2 or more months after harvesting. The Nancy Hall, Triumph, and Southern Queen varieties were tested only after they had been stored for some time.

The Nancy Hall variety was found to contain 30 units of vitamin A per gram, or 13,500 units per pound; the Triumph, 2 units per gram, or 900 units per pound; and the Southern Queen, 4 units per gram, or 1,800 units per pound.

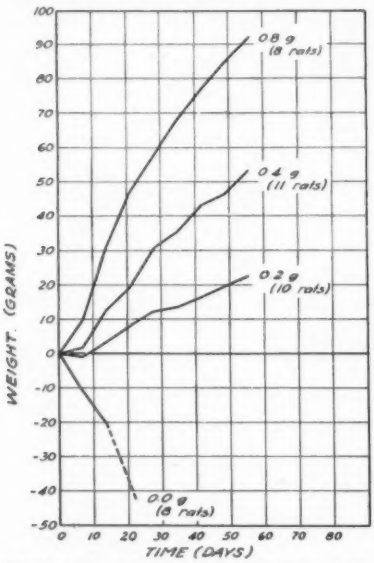

FIGURE 5.—Vitamin A content of the Southern Queen sweetpotato, showing average gain or loss in weight of rats fed this sweetpotato as their sole source of vitamin A. The amount of sweetpotato fed per rat per day and the number of rats in the group are shown at the end of each curve; broken lines indicate the death of one or more animals.

The Porto Rico variety was found to contain about 20 units of vitamin A per gram, or 9,000 units per pound, directly after harvesting. After the potatoes had been stored for 2 months or more, the usual period of storage before consumption, they contained about 65 units per gram, or 29,000 units per pound. The vitamin A value of these sweetpotatoes had increased, therefore, slightly more than three times.

Directly after harvesting, the Yellow Jersey sweetpotato contained less than 10 units per gram, or less than 4,500 units per pound. After storage for 2 months or more, the sweetpotatoes contained 40 units per gram, or 18,000 units per pound. The vitamin A value had increased in this case about four times.

Possible reasons for the increase in vitamin A during storage are given.

The Nancy Hall, Porto Rico, and Yellow Jersey varieties were found to be much richer sources of vitamin A than the Triumph and Southern Queen varieties.t Vitamin A values were found to vary with depth of color.

LITERATURE CITED

(1) CRIST, J. W., and DYE, M.
1929. THE ASSOCIATION OF VITAMIN A WITH GREENNESS IN PLANT TISSUE. II. THE VITAMIN A CONTENT OF ASPARAGUS. Jour. Biol. Chem. 81: 525–532, illus.

(2) FRAPS, G. S., and TREICHLER, R.
1933. VITAMIN A CONTENT OF FOODS AND FEEDS. Tex. Agr. Expt. Sta. Bull. 477, 34 pp.

(3) KRAMER, M. M., BOEHM, G., and WILLIAMS, R. E.
1929. VITAMIN A CONTENT OF THE GREEN AND WHITE LEAVES OF MARKET HEAD LETTUCE. Jour. Home Econ. 21: 679–680.

(4) MAGOON, C. A., and CULPEPPER, C. W.
1922. A STUDY OF SWEET-POTATO VARIETIES, WITH SPECIAL REFERENCE TO THEIR CANNING QUALITY. U. S. Dept. Agr. Bull. 1041, 34 pp., illus.
(5) MORGAN, A. F., and FIELD, A.
1930. VITAMINS IN DRIED FRUITS. II. THE EFFECT OF DRYING AND OF SULFUR DIOXIDE UPON THE VITAMIN A CONTENT OF FRUITS. Jour. Biol. Chem. 88: 9–25.
(6) RICE, P. B., and MUNSELL, H. E.
1931. THE APPROXIMATE UNITS OF VITAMIN A AND VITAMIN C IN FOODS. [6] pp. New York Association for Improving the Condition of the Poor.
(7) SHERMAN, H. C., and BURTIS, M. P.
1928. FACTORS AFFECTING THE ACCURACY OF THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Biol. Chem. 78: 671–680.
(8) ——— and MUNSELL, H. E.
1925. THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Amer. Chem. Soc. 47: 1639–1646, illus.
(9) STEENBOCK, H., and SELL, M. T.
1922. FAT-SOLUBLE VITAMINE. X. FURTHER OBSERVATIONS ON THE OCCURRENCE OF THE FAT-SOLUBLE VITAMINE WITH YELLOW PLANT PIGMENTS. Jour. Biol. Chem. 51: 63–76, illus.

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